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THE EFFECT OF CERTAIN MICRONUTRIENT ELEMENTS ON THE GROWTH AND YIELD OF PINEAPPLE PLANTS
by Francisco J. Ramírez-Silva—PAGE 197

CALCIUM–BORON RELATIONSHIPS IN THE NUTRITION OF CORN AND THE DISTRIBUTION OF THESE ELEMENTS IN THE PLANT
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I—INTRODUCTION *

The pineapple was, almost certainly, native of Brazil (5), and very probably the Caribs or Arawaks took some to Puerto Rico before the sixteenth century.

The pineapple (66) is related to the bromeliads and air plants, and it can absorb nutrient constituents through its leaf axils and long-barbed and barbless bayonetlike leaves that protrude from numerous whorls on the main stalk. The so-called pineapple fruit is an aggregate of many individual fruits with their fibrous juice pulp surrounding the core. Pineapple plants are propagated under field conditions from three asexually produced vegetative organs, known respectively as suckers, slips, and crowns. (See figure 1). In some varieties each individual fruit has a number of seeds which can be used for propagation but the most common practice is to plant slips or suckers. The slip resembles a miniature plant and is produced near the base of the fruit. The suckers resemble the slips, but are larger. The crown slip is produced at the top of the fruit; it is rarely used for propagation.

After one crop of pineapples is harvested a second crop, or ratoon, is produced from a new plant resembling a sucker which is formed at the base of the main stalk in contact with the soil.

Pineapple plants respond to good soil that must be well managed, not too alkaline or wet, and well aerated. The acreage in pineapples in Puerto Rico is relatively low compared to that in other crops. The total value of the export crop is high, although very low if compared with sugar cane. Practically all fresh and canned pineapples exported go to the United States where they are considered of good quality and get the highest prices.

The pineapple growers of Puerto Rico claim that the yields from native slips show a yearly lowering in production, as if they were degenerating or as a result of a "rundown" of the stock. Slips imported from Cuba when first planted in Puerto Rican soil, show considerably greater vigor than native slips. They give higher yields than the native stock brought originally from Cuba. It has been observed also that Cuban stock will show signs of decreased vigor and yield after a few years of propagation. Since pineapples are,

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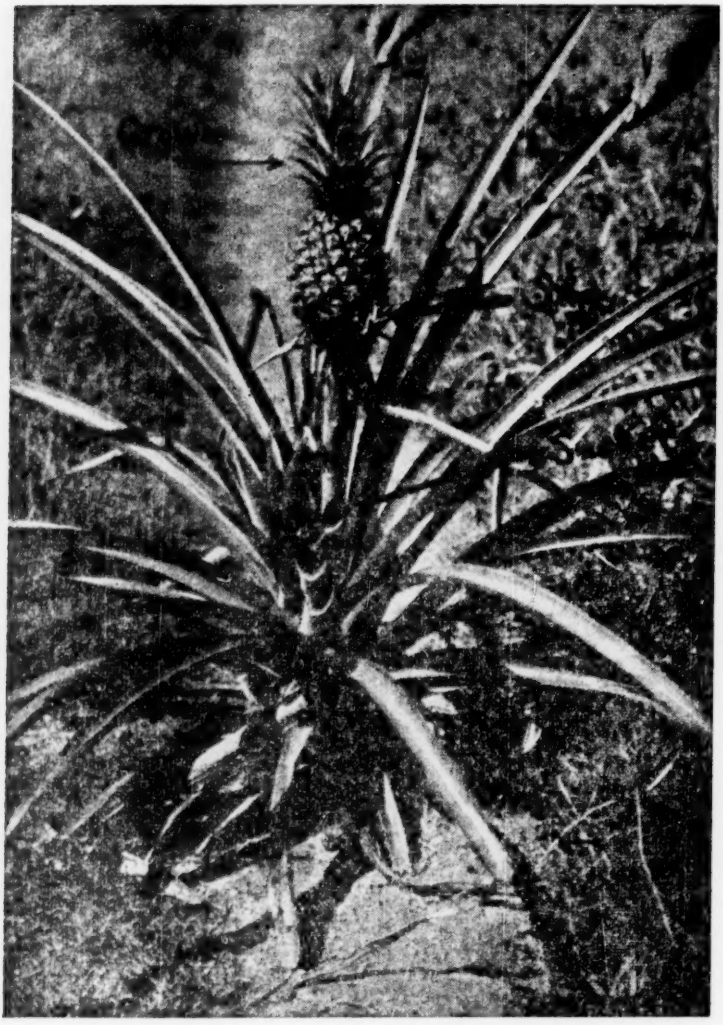


FIGURE 1.—A pineapple plant showing the position of slips, suckers and crown.

as explained above, propagated under field conditions from asexually produced vegetative organs, the degeneration is probably not on a genetic basis. On the other hand, no degenerative disease such as mosaic has ever been found. Hence, there is evidence of the possibility of a nutritional disturbance, perhaps in the micronutrient elements, that may produce vegetative organs with low reserve nutritional elements. These, on being planted in a soil causing such disturbance, may bring about the above-mentioned apparent degeneration of the stock.

This nutritional derangement may have been produced by changes in the soil as a result of faulty cultivation methods, fertilizer practice, constituents of the parent material of the soil, poor crop rotation and conservation. It is claimed, however, that slips planted on virgin soil or on soils not used before for pineapple planting, do not show this degeneration taking place so rapidly as on soils previously used for several years under continuous pineapple production.

Pineapple chlorosis has also been known in Puerto Rico from early times, as shown in works done by Gile (25) since 1911, and by Henricksen (31, 32) in 1925. Similarly, pineapple chlorosis was also known in Hawaii as shown in the experiments of Kelly (50, 51, 52, 53) from 1909 to 1914, also of Wilcox and Kelly (85) in 1912, and of Johnson (45, 46, 47, 48, 49) in 1916–1924.

This yellowing is a source of considerable loss. Spraying of the chlorotic pineapple plants with iron sulfate solution has been practiced in Puerto Rico and in Hawaii, this being an adequate remedy for this malady.

These problems were brought to the attention of Schapelle (67, 68, 69, 70, 71) in 1939, at the Agricultural Experiment Station of the University of Puerto Rico. He set up experiments in order to examine the effect of different nutrient elements under different treatments in the field and under different concentrations of macro and micronutrient elements in solution cultures. His investigations on the nutritional aspect of these pineapple problems in Puerto Rico were followed by the experiments of Hopkins, Pagán, and Ramírez-Silva (40). The experiments presented in this dissertation are part of this series of greenhouse and field investigations on the above-mentioned pineapple problems. The effect of the micronutrient elements: iron, manganese, aluminum, boron, copper and zinc, on pineapple growth and production was examined by means of different treatments in solution cultures.

In accordance with Hoagland (34) who considers the terms "minor elements," "rare elements," and "trace elements" as inappropriate, we use the term he suggested, that is, "micronutrient" elements. Among these are iron, manganese, aluminum, boron, zinc, copper, etc., called micronutrient elements because of the minute concentrations in which they are found in plants. Very minute quantities of them are required to perform their essential functions in plant nutrition. Also, small amounts of them are enough for plants to restore themselves from the specific abnormalities and impaired physiological functions caused by their deficiency in the nutrient medium.

In contrast with these "micronutrient" elements there is a group of elements once called "essential" elements and now usually called "major" elements in plant nutrition. Among them are: nitrogen, potassium, phosphorus, calcium and magnesium. As they are found in greater quantities, they will be referred to as "macronutrient elements." In this sense, the idea of either major or minor, or of essentiality, will not be erroneously conveyed. It may be the case that the so-called "minor elements" be of "major" importance in some cases of plant nutrition. No element is "major" or "minor"; they may be either macronutrient or micronutrient, according to their concentration in plants.

The artificial culture method has been found to be a valuable tool in plant nutrition research. This method has been termed "hydroponic," "water culture," "sand-culture," "gravel-culture," "solution-culture," etc. The term "water-culture" is widely used, but it is not as accurate as the term "solution-culture," since it is a solution of nutrient substances that is used in this artificial method. Of course, when sand or gravel is used in the culture media, the corresponding term of sand or gravel culture is appropriate.

II—REVIEW OF LITERATURE

A. The growth of plants in artificial media in relation to the study of plant nutrition

In order to study the role played by nutrient elements in the plant, and in an attempt to separate this from the problem of the availability of the nutrient elements in the soil, that is, from the soil-solution problem, the artificial culture method has come to be a valuable tool in this field of research.

The method of growing plants in nutrient solutions has been used as the best known means of controlling the concentrations, pH,

and proportions of nutrients fed to plants in experimental treatments. The convenience of this method, as well as the objections to it, depend upon the specific problem under experimentation.

Since the earliest recorded experiments with solution cultures by Woodward (61) in 1699, and later on by procedures developed by Sachs, Knopp, and Nobbe, from 1859 to 1865, the modifications introduced by means of different formulas and techniques have made this medium of growth for plants very useful for the purposes of fundamental experimentation and preliminary trials for field work and research.

Hoagland (34), on his discussion of the topic of growth of plants in artificial media in relation to plant nutrition, taking in consideration the dominating phases of this subject, appraises the far-reaching scientific significance of this method and its great service in understanding the nature of the soil-plant system.

According to Miller (61), nutrient solution formulas have been proposed by Tollens in 1882, Schimper in 1890, Pfeffer in 1900, Crone in 1902, Tottingham in 1911, Shive in 1915 (73), Hoagland in 1920, and many others. The osmotic pressure of the solutions has been considered, also, the relationship between proportion of salts, the growth effect of light and temperature, the relative absorption of ions, and the functions of the elements essential to plant life. Miller (61) shows a list of useful formulas for nutrient solutions.

Shive's (73) three-salt solution of KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$ has been the basis of much experimentation by him and others. They have considered the osmotic pressure best fitted to certain crops, the supply of essential elements, and the relation of the elements as found in soil extracts. The formulas derived from Shive's three-salt solution have been classified in series according to osmotic pressures and the molecular proportions of the dissolved components. They are designated by serial numbers when reference is made to them in experimental work.

For experimental purposes it has been recommended that renewal of solutions be made at least every month, and if possible continual removal, aeration by bubbling air, adequate temperatures, uniformity in light supply, and the use of pure chemicals and distilled water.

The works of Lowhwing (54) in 1932, of Clark and Shive (19) in 1932, and of Bryant (14) in 1934, show that nonaerated nutrient solutions tend toward a greater ratio of roots to tops. This suggests the idea of growing plants for a certain period of time, in the beginning without aeration, later on aeration is applied as required for normal crop production.

The preparation of separate solutions to prevent precipitation of concentrated salts upon being mixed was recommended by Tottingham (81) in 1914.

B. *Researches on nutrition of pineapple plants as related to micronutrient elements*

During the last two decades mineral deficiencies in plants have been the subject of much experimentation in the field of plant nutrition. Nevertheless, the question of toxicity due to excess of certain nutrient elements has not been emphasized to a great extent from the standpoint of practical plant culture. The balancing, additive, and antidoting effects between certain nutrient elements have been the subject of still less investigation. Examples of this toxicity are shown by manganese in pineapple plants, as reported from Hawaii by Kelly (50, 51, 52, 53) in 1908–1912, by Johnson (45, 46, 47, 48, 49) in 1916–1924, by Wilcox & Kelly (85) in 1912, and by McGeorge (58) in 1923.

In 1911–1916, Gile (25, 26) and Gile and Ageton (27) found a type of chlorosis of pineapples in Puerto Rican soils that contained large quantities of calcium carbonate and no excessive manganese. They called such chlorosis, lime-induced chlorosis. They noticed that spraying with solutions of iron salts resulted in the restoration of the green color. Johnson (45) in 1916, however, traced the pineapple chlorosis as due to manganese toxicity with a condition of low or no iron absorption, and found that spraying with iron sulphate solution counteracted the effect of high manganese present in the soil. On the other hand, Gile (25) found that this spraying of plants had to be repeated so often that it was not economically practicable.

As we are dealing with the general term "chlorosis", it must be explained here that it stands for a diseased or unhealthy condition of the leaves shown by the loss of the green coloring matter. This yellowing may be due to several factors such as lack of the nutriments producing or intervening, directly or indirectly, with the process of chlorophyll formation, to poor drainage or bacterial effects. After the leaves become strongly chlorotic necrosis ensues, showing extensive black areas.

Schapelle (67, 70, 71) in 1940, attempting to elucidate the problem of the apparent degeneration of pineapple stock and the increasing chlorosis of pineapple plants grown in Puerto Rican soils,

made studies on the effects of macro and micronutrient elements on the growth, yield, chlorosis, and quality of fruits of pineapple plants. He found that potassium was an important factor in the quality of the fruits, that plants responded better to ammonia than to nitrate nitrogen. He noticed that chlorosis was a cause for lower yields. He found profitable the use of frequent spraying with ferrous sulfate solutions. In experiments with pineapples grown in nutrient solutions he used with success a modified form of the solution used by Sideris, Krauss, and Young (74). He had demonstrations of best growth under the conditions of the experiment at a pH around 4.5, that manganese and zinc tended to produce chlorosis, that aluminum and boron tended to counteract the effects of manganese and zinc, and that copper, added at the concentration of 2 p.p.m. controlled a root fungus that caused stunting of the pineapple plants.

After this work of Schapelle (67, 70, 71), Hopkins and co-workers (40) in 1944 started their work along this line of research not only using the pineapple plants but also beans and tomatoes. They found: (*a*) high amounts of water-soluble manganese in soils from pineapple growing areas, (*b*) observed the chlorosis of pineapples grown in those soils, and that beans would not grow on them on account of severe chlorosis, (*c*) that in spite of the correction of chlorosis in pineapples by spraying with ferrous sulphate, certain abnormalities occurred, such as reduction in size of the plant and development of more red pigments than normally, (*d*) slips imported from Cuba, of the variety "Red Spanish," produced larger and greener plants than slips from the same variety from Puerto Rican plants, but after being planted for two generations in Puerto Rico, plants originally from Cuban slips reverted to the inferior type, (*e*) many fruits produced in these soils were affected with "short top," that is, a very short crown in the fruit which appears to be brought about by high soluble manganese under high intensity of light. The pineapple soils have pH as low as 3.8 which brings manganese into solution. Tomato plants used for preliminary experiments showed high correlation of maximum growth with the wider ratio of Fe to Mn. Tomato plants show a great sensitivity to manganese, requiring a large ratio of Fe to Mn for normal growth, flowering and production. It was noticed that the reserve of iron in seeds or propagating organs supplied the plant during a certain period of the early growth with the iron necessary to antidote the manganese in the nutrient solution, in either soil or solution culture.

C. *Researches on plant nutrition as related to micronutrient elements used in these experiments*

The micronutrient elements used in this experiment: iron, manganese, copper, zinc, aluminum and boron, have been the subject of an immense amount of research. To review and make a thorough study of all this literature would be outside the scope of this dissertation, but a chronological review of investigations having a bearing on this experiment is necessary. In the following pages such a review is made, taking in consideration that some references have already been mentioned in previous sections.

1. *Iron*

Iron is essential for the growth of plants. In them it is almost universally present in small quantities. Molisch in 1892, as quoted by Miller (61), found that most iron in plants is in a combined, insoluble form. Iron apparently occurs in plants in two forms, namely, the "active and inactive", sometimes also called "available and non-available".

Although plants deprived of iron show marked chlorosis, it has been definitely proved by Willstatter and Stoll in 1913 that iron does not enter into the composition of chlorophyll. Gile and Carrero (28, 29, 30), studied in 1914–1916 the iron requirements of rice, pineapples, and other crops. They noticed that iron, after being transported to the leaves, is immobile, and that colloidal iron is not absorbed by plants. Warburg (83) proposed in 1925 the theory that iron is the oxygen-carrying component of the respiration ferment. Hopkins (36) 1930, believed in the important role played by iron in the cellular processes involving biological oxidation.

According to Miller (61), the researches made by Oddo and Pollaci in 1920, Deuber in 1926, and Pollaci in 1935 show a possible explanation of this role of iron as a catalytic agent in chlorophyll formation, catalyzing the formation of the pyrrole nucleus which is the center of the chlorophyll complex.

Gile and Carrero (30) in 1920, and Willis and Carrero (87) in 1923, agreed in considering that lime-induced chlorosis is due to a depression in the available iron. Rippeal (65) observed in 1923, that chlorosis produced by manganese in the form of soluble salts in solution cultures, was overcome by increasing the supply of iron. He concluded that manganese interfered with the iron within the plant and not with its absorption.

Gericke (24) noticed in 1923 that iron-deficiency symptoms were more acute in plants under lights of high intensity than in lights

of low intensity. This should be examined according to the results of experiments made by Hopkins, Pagán and Ramírez-Silva (40) in 1944. They noticed phototropic effects of manganese in the absence or low level of iron. It is possible that Gericke was taking for iron deficiency what may be called manganese toxicity.

Burk, et al (16), made a report on the experiments made on metallic humates up to the year 1931 and, with reference to iron humate, they pointed out important facts that show the advantages in using humic acid for furnishing iron in a permanently soluble form in soils and in artificial culture media. The humic acid itself does not act by increasing directly the availability of constituents added to or present in the culture medium; or by activating toxic metabolic products; or by affecting surface tension, viscosity, and potential differences between culture medium and organism, or the oxidation potential of the media; but provides iron for growth and nutrition in a more highly available form.

Horner, Burk and Hoover (41) explained in 1934 a method of preparing humate metals from the salts of the corresponding metal and humic acid made from sucrose. This method is simple and provides a soluble form of iron at a very wide range of pH values. It is of great utility in solution cultures where available iron is required for treatments at different pH values, and also when the culture solution is subjected to variations in pH. These humates are stable in alkaline, neutral and moderately acid media, and are not precipitated by phosphates.

The role of iron in plant metabolism should be related to enzymes called iron enzymes, namely, catalase, peroxide, citochromes, indophenol oxidase, and others.

2. *Manganese*

Manganese is widely distributed in nature. It is an essential element for plant growth and functions in the synthesis of chlorophyll and carbon assimilation. According to McHargue (60), previous to 1774 the compounds of this element were confused with those of iron. In that year Scheele discovered that the metal found in pyrolusite, manganese, is altogether different from iron. Gauhn isolated the metal shortly afterwards. Thus Scheele started the work to investigate the functions of manganese in plant economy and its occurrence in soils. Ninety years later Sachs was able to prove that plants assimilate manganese and that it cannot replace the functions of iron in plant growth. By 1894 Bertrand had already determined the chemical composition of the sap of the lac tree and found that

its ash contained 2.5 per cent of manganese. The importance of manganese, in spite of the small content of it in plants, was by that time already recognized.

The works of McHargue, in 1914, showed that manganese and iron play an important role in chlorophyll formation and that this element may be toxic to plants at certain levels of concentration in the nutrient solution.

Sachs in 1865 noticed yellowing and etiolation of leaves in an attempt to substitute manganese for iron. Field observations on pineapples grown in manganiferous soils show that the interior of the fruits have a whitish appearance and usually contain excess acidity. Lime application makes manganese toxicity worse.

In 1908 Fukutome (22), from Tokyo, in his experiments on flax, noticed beneficial action when iron sulfate was added to manganese treatments. This is sometimes termed antagonism, counteracting effect, or antidoting action. Masoni (55) also observed in 1911, in his experiments with corn and lupines, the beneficial counteracting effect of iron on the detrimental action of manganese.

Stocklasa, J., (79) noticed in 1911 that aluminum and manganese have an additive beneficial effect on the growth of several plants.

Wilcox and Kelly (85) in 1912, in their experiments with pineapples, grown in Hawaii, made a thorough study of the physiology and chemistry of pineapples as affected by manganese toxicity. They do not mention the antidoting action of iron. They observed the mechanism of chlorosis, and the bad effects on roots due to excessive soluble manganese in the nutrient solution.

Kelley (53) in 1914 indicated that manganese may have an effect upon soil so as to bring about the mobilization of calcium and magnesium, and that it may stimulate the oxidation going on within the plant and in the soil.

The antidoting action of other cations as Ca, K, Na, and Mg on Mn was observed by McCool (56) in 1913. It may be that the beneficial effect of manganese is only due to the association or counteraction of other cations. He noticed that the deleterious effect of manganese varied inversely with the intensity of light.

Funchess (23) noticed in 1918 that nitrates and nitric acid increased the toxic concentrations of manganese.

Hopkins (36, 37) in 1930 reported the increase of growth of chlorella six hundredfold by addition of manganese to the nutrient solution, and he suggested that manganese functions in an indirect

manner in plants by its action upon the oxidation of iron. Hopkins (39) also noticed slow growth of *Lemna Minor* with iron and no manganese.

Bortner (11)) in 1935 observed chlorosis produced in tobacco plants by manganese in concentration of 15 p.p.m. in the nutrient solution, and noticed the antidoting effect of phosphorus. On the other hand, Sherman and Harmer (72) in 1941 found symptoms of manganese deficiency in oats manifested by specks and chlorosis which were prevented with the application of manganese.

Hopkins, Pagán and Ramírez-Silva (40) in 1944 found increase in growth of beans and tomatoes when manganese in soil was immobolized in the soil, and still more growth if iron humate was added. Marked detrimental phototropic action was noticed with excess supply of manganese, and in this effect, the antidoting action of iron was very effective.

Arnon (3), in his report of a review of the research done in mineral nutrition in plants for the year 1943, presents the following hypothesis offered by Somers and Shive: The cells of plants can tolerate only a certain definite concentration of iron which is of ferrous valence. The function of manganese is to regulate the concentration of ferrous ion. Manganese ions oxidize ferrous to ferric ions which precipitate in the form of "ferric-phosphorus organic complex." rendering iron physiologically inactive.

3. *Copper*

Copper is widely distributed in plants in considerable quantity, but according to research done it has stimulating action only at very low concentration, and it is generally toxic to green plants. The content of copper in pineapple fruits, according to an analysis shown by Beeson (8), is about 8 milligrams per kilogram. McHargue (59) reported in 1925 the copper content in various plants and plant parts as ranging from traces to 46 parts per million.

Plants respond to adequate copper treatments, as explained by Miller (61), showing increase in vigor, yield, quality, and control of chlorosis. He quotes the work of Maquene and Demossy in 1920 where they show that copper is found in greatest abundance in cells that are active in growth, and that its translocation is controlled by metabolic processes.

In the raw peat soils of the Everglades of Florida, Allison, Bryan and Hunter (2) in 1927 found copper to be a specific limiting factor, giving response in growth and production in a remarkable way when 30 and 50 pounds of copper sulphate per acre were applied to the soil.

Miller (61) gives a review of outstanding research done up to 1945 with copper and he points out the beneficial effect in some crops like onions, and in some fruit trees, when used as a fertilizer or as spray.

Hopkins (38) found in 1933 no increase in growth attributable to additions of copper to culture of *Lemna* and *Chlorella*.

Skoog (76) claims that copper may be related to the respiratory process.

The essentiality of copper for many species has been recently demonstrated by Hoagland and others, as reviewed by Petrie (64).

Felix (21) experimented in 1927 with onions and lettuce on the reclaimed muck lands of Central New York and found copper to be a limiting factor. Lack of copper produced a specific anatomical abnormality known as "rabbit ear". Onions fertilized with $CuSO_4$ produced better colored and thicker scales.

It should be noticed that according to the findings of Waddell and Steenbock (82) in 1929, copper is regarded as a necessary adjunct to iron in the regenerated of anemia in animals. This may be considered as parallel to its effects in chlorophyll regeneration. Schappelle (71) claims that copper at a rate of 2 p.p.m. in the nutrient solution controlled a root fungus that caused stunting of the pineapple plant.

The so-called copper enzymes and copper containing proteins should, in part, show the relation of copper to respiration and oxidation processes in plants.

4. *Zinc*

The effect of zinc on plant metabolism is one of the most interesting phases of the field of plant nutrition.

The essentiality of zinc in corn plants was recognized, according to Miller (61), by Maze in 1915, and by Somer (77) in 1928, who noticed abnormalities in the growth of buckwheat and sunflower, and in the flowering of *Vicia faba*.

Bertrand and Andreitcheva (9) in 1933 considered the zinc content correlated with a high chlorophyll content.

According to Miller (61) zinc deficiency caused the plant disease called "little leaf," as demonstrated by works of Chandler, Hoagland and Hilbard in 1933, on peaches, apricots, tobacco, squash, corn, mustard, tomatoes and other plants.

Mowry and Camp (62) in 1934 found that spraying with zinc sulphate, or its addition to soil, made tung trees recover from bronzing.

Chapman, Vanselow and Liebig (18) produced mottle leaf by omitting zinc from culture solutions. The "mottle leaf" disease in citrus orchards is caused undoubtedly by zinc deficiency. Hoagland (34) describes the fight against this disease in California during twenty years, and says that not a good clue to its cause has been found yet.

Spraying with appropriate zinc compounds, he says, is effective and commercially practical. In Florida, the disease on pecans called "rossette," in Australia a disease of pine trees, and in Hawaii pineapples showing distorted blades, are conditions remedied by zinc sprays. It has been observed that for various reasons zinc in the soil is sometimes not made available to plants. It might be fixed to soil colloids. Certain soil organisms are a recognized factor in the non-availability of zinc by competing with the plant. Some plants have more capability than others for absorbing zinc when it is in a low supply from nutrients. Certain plants like alfalfa show a high ability to absorb zinc from the nutrient media.

Hoagland (34) considers that the quantitative requirement of zinc, as well as the deficiency symptoms, are in part governed by climatic or seasonal factors. High intensity of light aggravates the zinc-deficiency symptoms. (This is an important factor in tropical agriculture.) According to Hoagland (34), and the work of Skroog (76) about this phototropic action of zinc, auxin formation in plants is connected with zinc nutrition. Auxin breakdown is promoted by short-wave light.

The translocation of zinc is effected through the breakdown of the zinc protein compounds under the action of reduced light, thus releasing the zinc which is transplanted to regions of active growth. So, zinc is directly or indirectly connected with protein synthesis in plants. As it does not undergo reversible valence changes, its action in oxydation-reduction systems, if any, must be an indirect one, or due to its influence upon oxidizing enzymes and its interrelation with iron. Hence, zinc is related to respiratory processes and the maintenance of normal concentration of auxin in tissues as claimed by Skoog (76).

Thatcher (80) believed that copper and zinc are mutually counterbalancing catalyzers for hydrogen exchange, as shown by their strikingly opposite effect upon reversible oxidation-reduction reactions of both glutathione and ascorbic acid.

Leaf chlorosis in grape fruit trees in Puerto Rico, resembling the "mottle-leaf" in California and the "frenching" in Florida, was successfully controlled by Jensen (44), at the Federal Agricultural

Experiment Station of Puerto Rico, by spraying with zinc sulfate solution. Pineapple, under certain conditions of zinc deficiency, according to Nightingale (63) in 1942, show characteristic spots or blisters from which they recover by spraying with zinc sulfate solution.

As quoted by Beeson (8), a pineapple fruit analysis showed 20 milligrams of zinc per kilogram. Willis (86) showed that during the last fifteen years experimentation on this micronutrient element has demonstrated the essentiality of it for the normal growth of green plants, and its deficiency as causing characteristic chlorosis, mottle leaf or frenching, and "rossete" or "little leaf" in fruit trees.

5. *Aluminum*

Aluminum is very abundant in soils. It has been found in all plants that have been analyzed but, as a rule, the percentage of aluminum in plants is very low. It may thus be considered a micronutrient element. Grains and vegetables analyzed by Meyers and Voegtlin, as quoted by Miller (61), contained from 0.045 per cent dry basis in wheat flowers, up to 0.996 per cent in cotton seed. As to the role of this element in the growth and production of plants, Miller (61) reviewed the works of Yamano in 1905, who found injurious effects caused by 0.2 per cent ammonium alum on wheat and rye grown in nutrient solutions, and 0.8 per cent to be a fatal dose. Prianishnikov, in 1911, grew wheat, oats, barley, peas and buckwheat in sand cultures fertilized with aluminum phosphate and calcium carbonate alone. Baguley found in 1912 the iron and aluminum phosphate combination to be better. Kratzman observed in 1914 toxic effects of 0.005 per cent concentration of aluminum salts. Others, in experiments done after these, have found toxicity of aluminum salts at certain low levels of concentration in the nutrient media.

The soil-plant aspect of aluminum has been studied more than others. In fact, considering soil work, aluminum shows a great complexity in relation to other elements, it being one of the principal components of soils and soil colloids. Its availability is greater at lower pH.

Schapelle (71) showed beneficial action and recovery from injuries brought to pineapple plants grown in nutrient solutions lacking in aluminum. But Abbot (1) found in 1913 aluminum to be a toxic agent in the marsh regions of peaty sand, and also in culture solutions.

Barnette (6) in 1923, using solution cultures and upon observing the toxic effects of aluminum ions, determined that such toxicity was not due to acidity per se, but to the hydrolysis of aluminum salts.

The function of aluminum within the cell of the plant is ignored yet. Miller (61), reviewing the work of Fluri in 1907, mentioned the consideration that aluminum has an indirect effect in starch disappearance from the cell by increasing protoplasm permeability, diastatic action, and slowing photosynthesis.

Stocklassa (79) found in 1911 that aluminum and manganese together stimulated growth of several species of plants.

Blair (10) observed in 1923 the detrimental effect of soluble aluminum in soils upon roots. McGeorge (57) reported in 1925 that he noticed toxidity of aluminum on roots, in culture solutions, at the pH of acid soils. Haas (35), nevertheless, observed beneficial effect of aluminum in solution cultures of lemon, leafy-twig cutting, when a good supply of phosphorus is present.

Burgess (15) determined, 1923, the availability of aluminum in some soils. At pH 4 to 5 he found 388 parts per million while at pH 5 to 5.8 it was lowered to 36 parts per million.

Arnon (3), in a review on plant nutrition for the year 1945, says that Liebig, Vanselow and Chapman claim that they found that aluminum at low concentrations counteracts copper toxicity in citrus grown in culture solutions. It seems, according to them, that the case of the beneficial action of aluminum depends on its action against the toxicity of copper.

At high concentrations (2.5 to 5 p.p.m.) aluminum gave a curious stimulation of root growth accompanied by depression of top growth. In the absence of aluminum, excessive copper caused a brownish appearance in citrus roots and short swollen laterals which gave the roots a dwarfed, knotty, and unhealthy appearance. Top growth often exhibited iron chlorosis.

6. *Boron*

Boron is the micronutrient element that has received the greatest attention; and still the mechanism of the function of this element in plant growth is hidden to us. As claimed by Chapman (17), up to now we have not passed beyond the knowledge of the effects of its deficiency upon the meristematic tissue, and its interrelation with calcium. There is a marked similarity between the symptoms of calcium and boron deficiencies. Boron is widespread in the plant kingdom, it probably occurs in all green plants. Since 1857 its

presence was detected in plants by Whittstein and Apogier (88), followed by Baumert (7) in 1888, Hotter (42) in 1890, and Jay (43) in 1895, who analyzed various plants and believed in the universality of boron in the plant kingdom.

The study of the influence of boron upon plant growth called the attention of many investigators. Sand, solution, and soil-culture experiments were made by Augulhon (4) in 1910 and by Brenchley (12) in 1914. Their findings point toward the beneficial effect of boron when supplied to the plant in the right amount. The work of Warington (84) in 1923 on boron compounds on beans, in solution cultures and field experiments, was the beginning of the consideration of the mechanism of boron nutrition in plants. She pointed to the catalytic action and its effect on meristematic tissues.

The effect of boron nutrition on nodule formation in leguminous plants was studied by Brenchley and Thornton (13) in 1925. They found beneficial action on the production of nodules as due to the anatomical conditions of the plants with good boron nutrition. From there on, the boron requirements for nutrition of many crops have been studied, as well as the symptoms of deficiency and toxicity for different plants.

Miller (61) reviews the studies made on boron deficiency, and from the works of Warington in 1926, points out that disintegration of phloem and ground parenchyma, poor development of the xylem, and hypertrophy, discoloration, and disintegration of cambial cells occur when boron is omitted from the nutrient solution. Growth is arrested in the meristematic tissues of root tips, as found by experiments of Sommer and Sorokin in 1928. The effects of boron deficiency in tomato plants, "the guinea pig of the plant nutritionist," are very noticeable: death of terminal growing points of stem, characteristic brittleness of the stem and petiole, and poor brownish roots.

That the function of boron cannot be performed by other elements was found by Warington in 1927. She tried fifty-two other elements. Sugar beet and alfalfa are plants very much affected by lack of boron, and show specific deficiency symptoms. Boron, indeed, will show harm on plants when supplied in excess, bringing about chlorosis. This is probably due to its action against the solubility of iron, as claimed by Rodríguez in 1935. (66a) An effect of boron toxicity is stimulation of undifferential cell division causing abnormal growth in the regions of its maximun effect.

The essentiality of boron for higher plants is no longer open to dispute. Brenchley (12) and Warington (84) proved that boron

is absolutely indispensable for satisfactory growth of many crops. Both report on the retardation of the development of meristem tissues and discoloration of the stem in plants as specific symptoms of boron deficiency.

Eaton (20) made in 1944 careful observations on the nutritional effects of boron, and noticed that in most plants it accumulates in soluble but largely immobile form. He suggests that boron becomes attached to some large molecule which, though soluble, is unable to pass through the plasma membrane of the mesophyll cells. Owing to this immobility of boron in leaf tissue, plants may show symptoms of boron excess in old leaves, and yet not be supplied with excess. Thus, there may be an overlapping of beneficial and toxic effects in the same plant. High-light intensity may be responsible for its immobility in the leaves. Boron deficiency is aggravated by increase of calcium in the nutrient solution, but its toxicity is lowered. Variations in potassium concentration affect indirectly boron deficiency and toxicity due to the effect of potassium on calcium absorption.

III — OBJECT OF THE WORK

It is the object of these experiments to study:

1. The effect of iron, manganese, zinc, copper, aluminum and boron on the growth and production of pineapples.
2. The antidoting effect of iron on manganese toxicity.
3. The mutual action of these micronutrient elements, and their deficiency or toxicity as affecting root growth, leaves, flowering, fruiting, yield, and quality of the crops.
4. The causative agents of pineapple chlorosis.
5. To verify the data already obtained in other experimentation in this field.
6. To guide future experimentation with pineapple plants along this line.
7. To suggest possible methods to remedy injuries on the pineapple plants as caused by malnutrition of the plant.

IV — EXPERIMENTAL

A. General Plan

Solution-culture methods were used in this experiment with a formula of macronutrient elements already found to be good for growing pineapple plants. The facilities of the greenhouse and hydroponic equipment of the Agriculture Experiment Station of the University of Puerto Rico (Fig. 2 and 3) as designed by Schapelle (71) were used. These experiments are a part of a research project of this Station.

The experiments consisted of two series of treatments. One series had nine treatments of micronutrient elements, to study the individual effect of the micronutrient elements when added to the culture solution. Treatment number one had no micronutrient elements added. This treatment showed the combined effect of all the reserve micronutrient elements in the planted slips. It served as a check on the other treatments. Treatments 2, 3, 4, 5, 6, and 7 corresponded, respectively, to additions of iron, manganese, boron, copper, zinc, and aluminum as the only micronutrient elements added to the nutrient solutions. These showed the beneficial effect of the presence of these elements, or their toxic effect, when acting independently at the concentrations added, as compared with treatment 1, to which no micronutrient elements were added. The eighth treatment contained all the micronutrient elements in the concentrations used in the previous treatments. This treatment was another check on the other six treatments. Treatment 9 was the same as 8 except that copper was not added. This showed the effect of lack of copper and was intended to check the results on root injury shown by lack of copper in the experiments of Schapelle (71). This treatment may be used to examine the effect of copper added in treatment 5 and, from this, infer its effect shown in treatment 8 where all micronutrient elements were added, and those of the following series.

Treatments 1 to 9 were designated as follows:

Treatment 1 as –ME (no micronutrient elements added)
Treatment 2 as Fe (iron added)
Treatment 3 as Mn (manganese added)
Treatment 4 as B (boron added)
Treatment 5 as Cu (copper added)

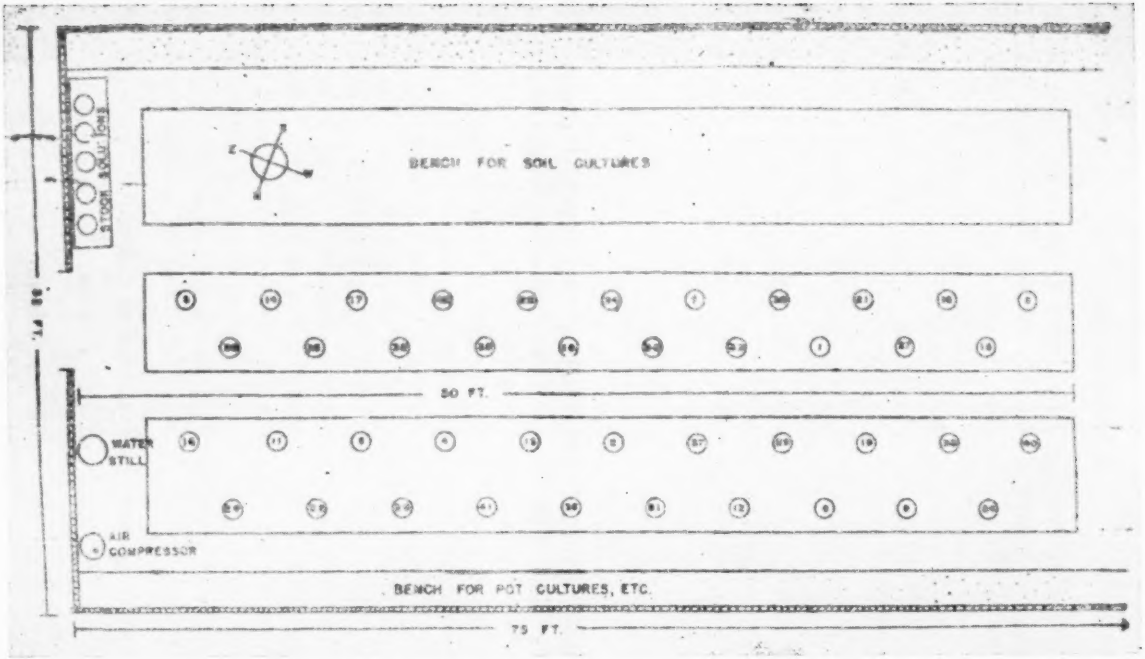


FIGURE 2.—Floor plan of the greenhouse showing the arrangement of the solution cultures. The numbers and position of the pineaple plants are shown within the circles.

Treatment 6 as Zn (zinc added)
Treatment 7 as Al (aluminum added)
Treatment 8 as ME (all micronutrient elements added)
Treatment 9 as –Cu (all micronutrient elements minus copper)

The series of treatments 10 to 14 was intended to study the antidoting action of iron against the chlorosis-producing effect of manganese. For this purpose 5 p.p.m. of manganese were added to each one of the five treatments, and a different level of concentration of available iron was added in each. So, treatment 10 had no iron added, number 11 had one p.p.m. added, number 12 had three p.p.m., number 13 had five p.p.m., and number 14 ten p.p.m. All these treatments were supplied with 2 p.p.m. of copper to prevent root injury as reported by Schapelle (71). The micronutrient elements: boron, zinc, and aluminum, were added also, in a concentration of one half p.p.m. each, in order to prevent deficiency of these elements. Treatment 2 with 5 p.p.m. Fe added as the only micronutrient element may also be considered as a member of this series for the purpose of the study of the antidoting effect of iron. The study of the results of the treatments 1, 3, 4, 5, 6, 7, 8, and 9 will throw light on the study of the series of treatments 10 to 14 and viceversa.

The effect of aeration of the culture solution was studied. During the initial period of growth the solutions were not aerated.

For the purpose of this study, observations and data on roots, plant growth, chlorosis, flowering, fruiting, yield, and quality of fruit were taken.

B. Methods

1. *Nutrient solutions*

Pineapple slips of the variety "Smooth Cayenne," 12 inches long and selected for uniformity, were "planted" in culture solutions. All the treatments contained the following concentration of macronutrient elements:

SALT	*Grams per liter*
$K H_2 PO_4$	0.1316
$Mg SO_4 7H_2O$	0.4100
$Ca(NO_3)_2 4H_2O$	0.4720
$NH_4 NO_3$	0.1260
K_2SO_4	0.1657

which furnish:

112.1 parts per million of K
30.0 parts per million of P
40.5 parts per million of Mg
80.1 parts per million of Ca

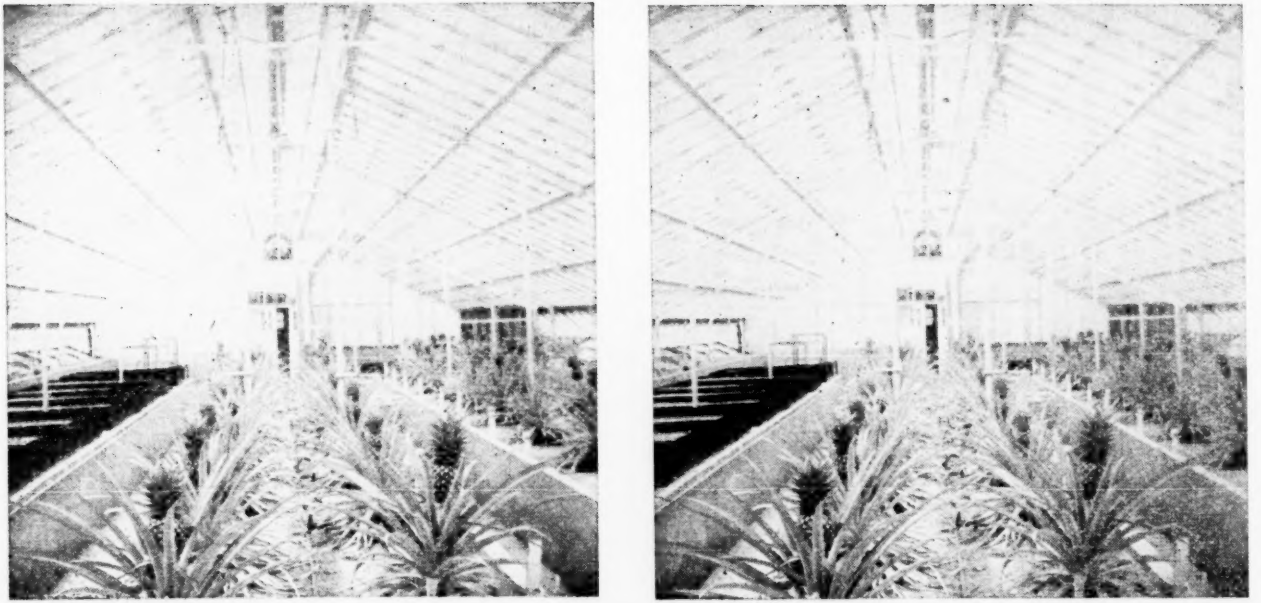


FIGURE 3.—Photographic views of the Greenhouse and the Solution Culture Experiment. Pineapple Plants, 493 days after planting. The air supply system for aerating the solution is shown.

83.6 parts per million of S
77.9 parts per million of Nitrate N
22.0 parts per million of Ammonia N

The following concentrations of micronutrient elements were added to the different treatments:

TABLE No. I

TREATMENT			PARTS PER MILLION					
No.	Elements	Jar Number	Mn	Cu	Al	B	Zn	Fe as $FeSO_4$
1	All M. E.	1- 2- 3						
2	Fe	4- 5- 6						5
3	Mn	7- 8- 9	2					
4	B	10-11-12				1		
5	Cu	13-14-15		2				
6	Zn	16-17-18					2	
7	Al	19-20-21			1			
8	All M. E.	22-23-24	2	2	1	1	2	5
9	Cu	25-26-27	2		1	1	2	5

THE IRON-MANGANESE SERIES

No.	Elements	Jar Number	Mn	Cu	Al	B	Zn	Fe as humate
10	5 ppm Mn.	28-29-30	5	2	½	½	½	0
11	5 ppm Mn Fe 1 ppm.	31-32-33	5	2	½	½	½	1
12	5 ppm Mn Fe 3 ppm.	34-35-36	5	2	½	½	½	3
13	5 ppm Mn Fe 5 ppm.	37-38-39	5	2	½	½	½	5
14	5 ppm Mn. Fe 10 ppm.	40-41-42	5	2	½	½	½	10

The solutions of micronutrient elements were prepared as follows:

Element	Parts per Million of the Element	Salt used C. P.	Grams of Salt per Liter
Mn	2	$MnSO_4 H_2O$	0.00615
Cu	2	$CuSO_4 5H_2O$	0.00785
Al	1	$Al_2(SO_4)_3 18H_2O$	0.012355
B	1	$K_2B_4O_7 5H_2O$	0.007476
Zn	2	$ZnSO_4 7H_2O$	0.008796
Fe	5	$FeSO_4 7H_2O$	0.02489

In every case stock solutions were prepared, so as to furnish the requisite amount of micronutrient elements, by using a definite adequate aliquot for the corresponding jar used in each treatment. The solution of ferrous sulphate was prepared in small quantities, at the required moment, to be used immediately. It was slightly acidified with sulfuric acid to prevent hydrolysis. Distilled water and C.P. chemicals were used always.

The iron humate added to treatments 10 to 14 was prepared according to the method of Horner, Burk and Hoover (41), and taking into consideration the recommendations of Burk, Lineweaver, Horner and Allison (16).

The macronutrient formula used in these experiments is a modified form of the solution used by Sideris, Krauss, and Young (74). It was used successfully by Schapelle (70, 71) in his work with pineapple plants.

The pH values of the nutrient solutions were determined periodically, by means of a Leeds and Northrup Universal glass electrode potentiometer. A pH value near to 4.5 was maintained by adding the required amounts of a normal H_2SO_4 solution or a dilute solution of $Ca(OH)_2$, according to the change in pH of the nutrient solutions. According to Schapelle (71), pH 4.5 is the optimum value for growth of pineapple plants in culture solutions.

2. *Method of growing the pineapple plants.*

The pineapple slips selected for these experiments were planted in quart culture jars of good-grade white glass which were prepared with suitable wooden lids with a hole in the center big enough to hold the slips. Three cultures were planted for each of the fourteen treatments, so, there were forty-two culture jars. These were distributed at random in the greenhouse. The arrangement and number of the treatments are shown in figure 2. Two photographic views of the experiments, when the plants were already fruiting, are shown in figure 3. The jars were buried in sand to exclude light rays from the solutions.

Table I shows the concentrations of micronutrient elements used for each treatment and the jars numbers.

The macronutrient solution used was the same for all the treatments. Its concentration is shown on page 216. The solutions were changed every month. The concentration of the solution of macronutrient elements was reduced to one half from the 214 day on.

On the 84 day after the slips were planted, the plants were transferred from the quart jars to wide-mouth gallon jars because the root system had grown already too big for the quart jars. For the same reason they were again transferred to 17-liter pyrex jars on the 214 day. The lids of these jars were made of wood and the center holes were lined with cork rings to hold the plants in place.

The roots were carefully drained of the residual solution in them every time that the solutions were changed. To change the solution, the plant was removed together with the lid and the jar was emptied

with a siphon. It was then cleaned of any solid residue and of the old solution. Then the jar was filled to about one half its volume with water. The corresponding aliquots of the stock solution of nutrient elements were added independently one by one, dissolving each one after added. The volume was completed to the 17-liter mark, and the plant put back to its place. The solution was brought to the 17-liter level with distilled water every day.

While the plants were growing in the quart jars, that is, during the first 83 days, the solutions were not aerated. From the 84 day on air was bubbled continuously through the solution. The air bubbles were passed at a uniform rate through all the jars, as exactly as possible. An automatically-pressure-controlled electric air compressor furnished the supply of air. Glass tubing was used to pass the air from the main air pipe into the solutions (see Figure 3).

The greenhouse, where this experiment was set up, was well ventilated by means of an electric fan located near the roof at one end of the building. (See Figure 3).

3. *Observations and data to be obtained.*

The plants were observed every day to note: (*a*) any change in conditions of growth, (*b*) root volume and development, (c) date of appearance and magnitude of chlorosis, (*d*) necrosis in leaves, (*e*) intensity of greenness, (*f*) condition and pH of solutions, (*g*) date of the beginning of blooming and of attainment of mature fruit, (*h*) weight and size of fruit and crown when harvested, (*i*) analysis of the juice of the fruit ripened out of the plant, for reducing sugars, total sugars, total acidity and density.

4. *Chemical methods of analysis.*

(*a*) *Extraction of juice*

The ripe pineapple fruits were carefully peeled from the non-edible rind, and the whole fruit cut in pieces and subjected to 500 pounds pressure per square inch in a Carver laboratory press. The juice expressed from the whole pineapple fruit was collected for analysis.

(*b*) *Density determination*

The degree brix of the juice was determined using brix hydrometers calibrated at 20°C. The readings made were corrected to 20°C, using the scale for corrections attached to the thermometer within the body of the hydrometer.

(c) Determination of reducing sugars

The official method (75) for the determination of reducing sugars and total sugars was used, that is, Lane–Eynon general volumetric method, as described in section XXXIV 32 of the Official and Tentative Methods of Analysis (75). This method uses Soxhlet's modification of Fehling's solution, as reagent. Solution (A) consists of 34.639 grams copper sulfate ($CuSO_4$ $5H_2O$) dissolved in distilled water and diluted to 500 ml., filtered through prepared asbestos in a Cooch crucible. Solution (B) is the alkaline tartrate solution, and is made by dissolving 173 grams of Rochelle Salts and 50 grams of sodium hydroxide in distilled water and diluted to 500 ml., then allowed to stand for two days and filtered through prepared asbestos. This solution was standardized according to the procedure in Section XXXIV, 33 of the Official Methods of Analysis (75), taking 5 ml. of solution (A) and 5 ml. of solution (B).

The determinations were made on a portion of the freshly extracted juice diluted 1 to 10. A one-per-cent-aqueous solution of methylene blue was used as the internal indicator. A preliminary incremental titration was made on the first sample, and the determinations made in triplicate, according to Section XXXIV, 34 (75), following a similar procedure to the one used for standardization of the reagent.

The calculation of the reducing sugars was made using Table XV Section XLIII of the Official Methods (75), taking the factors in the column for solutions having one gram of sucrose in 100 ml. of the juice solution used for titration. Of course, the readings of the volume in ml. of solution required to react to the end-point with the amount of reagent used, was divided by the concentration factor of the reagent's solution, to obtain the ml. that would be required for a reagent with a concentration factor of 1.0.

As the values thus obtained give the grams of reducing sugar in 100 ml. of the solution of juice used in the titration, multiplying these weights by ten will give the grams of reducing sugar in 100 ml. of juice. To obtain the percentage of reducing sugars weight in the juice, the weight in grams of reducing sugars in 100 ml. of juice is divided by the specific gravity 20°/4°C of the juice as determined from the brix reading, from Spencer (78).

(d) Determination of Total Sugars

Total sugars were determined promoting total inversion by adding 2 ml. of concentrated HCl (specific gravity 1.1029 at 20°/4°C)

to 10 ml. of the freshly extracted juice in a 100 ml. volumetric flask, left overnight for total inversion. Then the solution was neutralized with NaOH and brought up to the 100 ml. volume. From this, 10 ml. were diluted to 50 ml. and analyzed for reducing sugar by the same method given above for the fresh uninverted juice. The weight in grams of invert sugar in 100 ml. of the titrated solution of juice as calculated from the factors of Table XV, in the Official Methods (75) on the first column for no sucrose, must be multiplied by 50 to account for the two dilutions made: 10 to 100 and then 10 to 50. This gives the weight in grams of total sugars as invert sugar in 100 ml. of fresh juice. This value divided by the specify gravity 20°/4°C gives the percentage by weight of total sugars, as invert sugar in the juice.

(*e*) *Total Acidity*

The total acidity was determined by titrating the freshly extracted juice against a standard NaOH solution, taking 10 ml. of the juice. Phenolphthalein was used as the indicator.

According to E. K. Nelson of the Bureau of Chemistry, U.S.D.A., quoted by Hericksen (33), citric acid constitutes about 87 per cent of the total acids of pineapple juice. The acidity of the juice is calculated on the basis of per cent of citric acid in juice. This is done by multiplying the normality of the acid in the juice by one tenth of the gram equivalent weight of citric acid, that is, 6.4, and dividing by the specific gravity 20°/4°C of the juice so as to reduce it to gravimetric basis.

C. *Results and Discussion of the Effect of Micronutrient Elements.*

1. *Chlorosis*

The observations on the chlorosis shown by the plants, are expressed numerically in table II, indicating the total severity of chlorosis per treatment of three plants, the day of appearance, and of any change in the severity.

The numerical evaluation has been made on the following scale of values per plant:

Slight chlorosis	1.0
Medium chlorosis	2.0
Strong chlorosis	3.0
Very strong chlorosis	4.0
Necrosis	5.0
Very strong necrosis	6.0
Death of each plant	8.0

TABLE No. II

OBSERVATIONS ON CHLOROSIS OF PLANTS EXPRESSED NUMERICALLY FOR THREE PLANTS

Number	Treatment	Days after planting													Weighted averages 414 days
		88	90	94	97	101	107	111	119	132	143	170	214	414	
1	M. E.					1	2	2	3	4	5	6	6	6	4.2
2	Fe														0
3	Mn	1	1	2	2	3	5	6	6	6	10	10	10	10	7.1
4	B			1	1	2	2	3	3	4	5	8	8	8	5.3
5	Cu			1	1	2	3	3	6	9	14	14	14	14	9.2
6	Zn					1	1	2	2	4	4	5	5	5	3.5
7	Al			1	1	1	2	2	3	3	3	4	4	4	2.6
8	M. E.														0
9	Cu											1	1	1	0.6
	IRON-MANGANESE SERIES -5 PPM OF MANGANESE														
10	Fe 0 ppm	3	3	6	9	12	32	12	12	12	16	18	18	18	13.0
11	Fe 1 ppm														0
12	Fe 3 ppm														0
13	Fe 5 ppm														0
4	Fe 10 ppm														0

BASIS OF NUMERICAL EVALUATION PER PLANT

slight chlorosis	1.0	very strong chlorosis	4.0
medium chlorosis	2.0	necrosis	5.0
strong chlorosis	3.0	strong necrosis	6.0
	dead plant	8.0	

See Table 1 for concentration of micronutrient elements in each treatment.

For the purpose of comparison on the chlorosis produced by each treatment, averages have been calculated as "weighted averages". The magnitude of the severity is multiplied by its duration in days, and each summation of these products is divided by 414, that is, the number of days from the time of planting to the time of blooming of the plants.

The following method was used, taking treatment three as an illustration.

According to table II, in this treatment chlorosis appeared on the 88 day.

The chlorosis value	1	lasted	6 days
The chlorosis value	2	lasted	7 days
The chlorosis value	3	lasted	6 days
The chlorosis value	5	lasted	7 days
The chlorosis value	6	lasted	29 days
The chlorosis value	10	lasted	271 days

Multiplying each value of intensity of chlorosis by the number of days it lasted, then this gives:

		Without chlorosis	88 days
1	multiplied by	6 equals	6
2	multiplied by	7 equals	14
3	multiplied by	6 equals	18
5	multiplied by	7 equals	35
6	multiplied by	29 equals	174
10	multiplied by	271 equals	2710
Total "chlorosis days"			2957
Total number of days 414			
Average per day (three plants)			7.1

The average is calculated on the total growing period of 414 days. Some treatments showed chlorosis before others. Averaging for the total period of growth takes this into account.

Apart from the information given in table II, the following observations were made:

a. Treatments 2, 8, 11, 12, 13, 14, that is, all the treatments containing iron, showed plants with a good green color, especially treatment 2 that had iron as the only micronutrient element added.

b. The three plants of each treatment showing chlorosis were not affected equally in each case, some showed very slight chlorosis too low to be evaluated. The variations in reserve iron in the planted slip may be the chief reason

for this. The table gives information about the starting date, intensity in each case, and the rate of increase.

c. One plant in treatment 10 never bloomed and finally died of an extremely strong necrosis.

d. One of the plants of (copper) treatment number 5, showed also strong necrosis but was able to survive and bear fruit.

e. Attention should be given to the fact that treatment 3 had only two parts per million of manganese, while number 10 had five parts per million and the other micronutrient elements.

f. The degree of greenness in treatments 11 to 14 increased with the increase in iron in these treatments, but was not as high a green color as in number 2 that had iron as the only micronutrient element added. (Treatments 10 to 14 had other micronutrient elements added besides the manganese and iron. See Experimental Methods).

Discussion of the results on the chlorosis produced

All the treatments with iron added: 2, 8, 9, 11, 12, 13 and 14, were exempt from chlorosis.

Treatment 2, with iron as the only element added at 5 ppm, was the greenest of all treatments. Treatment 1, with no elements added, showed chlorosis; so, the iron added in treatment 2 was enough to prevent the chlorosis caused by chlorosis-producing elements in reserve in the planted slip. In other words, the reserve iron in the slip was not sufficient to counteract the chlorosis-producing tendency of the other elements through the whole period of growth. Extra nutrition of iron was required, as shown in treatment 2, to prevent chlorosis. Treatment 2 was greener than treatment 8 where the other elements were added and in which still no chlorosis was produced.

Treatments 10, 11, 12, 13, and 14 of the iron-manganese series, showed more definitely the counteracting action of iron against the chlorosis-producing action of manganese, and possibly of the other elements added in this treatment. While treatment 10 showed marked necrosis (one plant could not survive); treatment 11 (only one part per million of iron added) produced green chlorosis-free plants. Treatment 10 had five parts per million of manganese added and other micronutrients in smaller concentrations. (See Experimental Methods.) As will be shown later, these other elements have some tendency to produce chlorosis, especially copper.

Figure 4 shows plants under treatments 10 and 11, on the 102 day after planting. Notice that the plant of treatment 10 was strongly chlorotic, and that of treatment 11 was normal. Figure 5

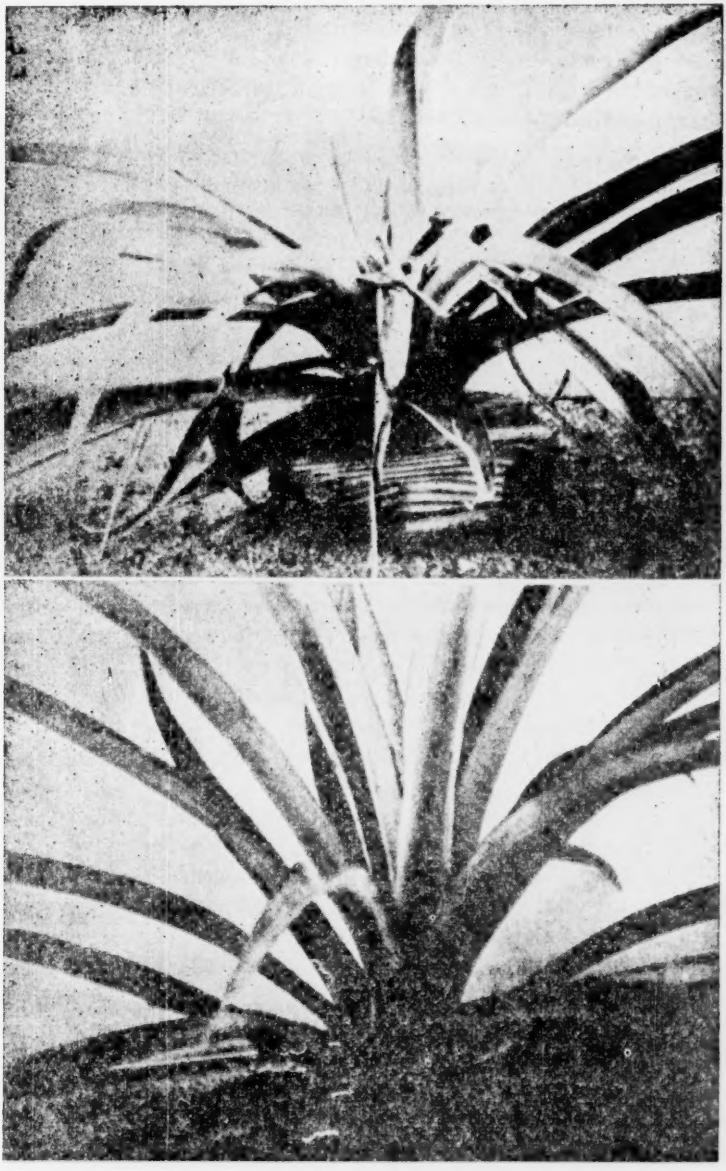


FIGURE 4.—Photographs of plants, 102 days after planting. Treatment 10, above. Micronutrient elements added to the culture solution: 5 ppm of Mn, 2 ppm of Cu, ½ ppm of B, ½ ppm of Zn, and ½ ppm of Aluminum. The plant is strongly chlorotic and necrotic.

Treatment 11, below. The same micronutrient elements as above but with one ppm of iron added. The plant is growing normally.

shows plants of the same treatments 493 days after planting. The plant of treatment 10 was almost dead and that of number 11 produced a normal fruit with a beautiful crown, a good sign of vigor and good quality.

The appearance of treatments 11, 12, 13, and 14, showed an increase in greenness with the increase of iron. No detrimental effect due to iron was noticed in any one of the treatments where iron was added.

The chlorosis produced by toxic concentrations of iron mentioned by Arnon (3), and the necessity of certain concentration of manganese to bring about a balance of the ferrous and ferric ions for proper chlorophyll formation, are not shown in these treatments. It may be that in the case of treatment 2, where iron was the only element added in five parts per million, the concentration was low enough, or the reserve manganese in the slip was at the right level; but in any case, there were no signs of iron toxicity. There are good reasons to believe that the toxicity of iron, if any, would be at a very high level in pineapple plants.

Treatment 5, that had copper as the only minor element added, showed strong chlorosis and even necrosis. This points out the conclusion that copper produces chlorosis at certain levels of concentration in the nutrient solution, if in the absence of iron. These results show the action of copper stronger even than that of manganese, which in treatments 3 and 10 induced chlorosis very markedly and in proportion to its concentration in the nutrient solution. See figure 6.

Boron also produces chlorosis when added to the nutrient solution in the absence of iron, or in the presence of insufficient iron to antidote its action. Compare treatment 4 with 1.

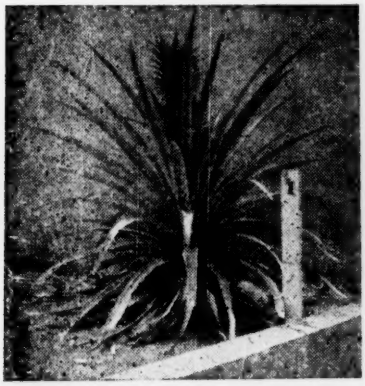
The above conclusions can be shown numerically as follows:

The average of the "weighted averages" of chlorosis of all treatments from 1 to 9 having iron, i.e., treatments 2,8,9, is 0.2; while the average for all treatments 1, 3, 4, 5, 6, 7 with no iron added, is 5.3. Also the chlorosis produced by treatments 1, without micronutrient elements added; 3 with manganese; 4 with boron; 5 with copper; and 6 with Zn; make a total of averages of 31.9. This was antidoted by 5 p.p.m. of iron added in the treatment 8, value 0, in which all the micronutrient elements were added.

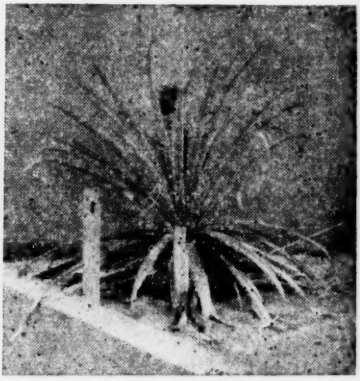
Evidence is given to definitely establish that, in the pineapple plant, iron antidotes the action of the chlorosis-producing elements copper, manganese and boron, when these are in a toxic concentration in the nutrient solution.

The treatments of zinc and aluminum showed beneficial effect against chlorosis. Treatments 6 and 7 gave lower values of chlorosis than treatment 1.

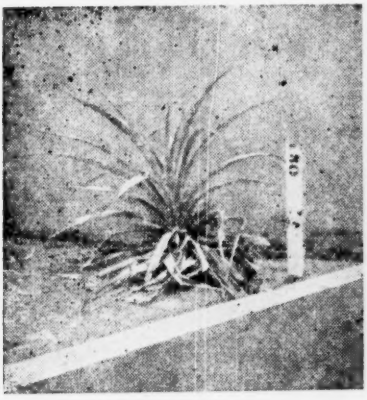
PINEAPPLE PLANTS, 493 DAYS AFTER PLANTING



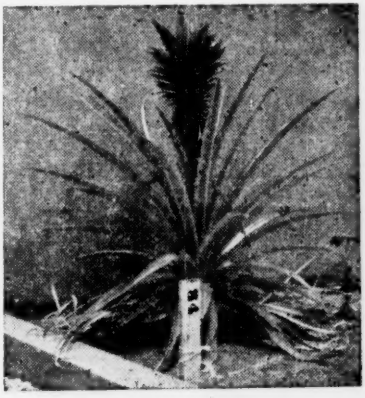
A. Treatment 1.
No micronutrient elements added.



B. Treatment 3.
2 parts per million manganese added.



C. Treatment 10.
No iron added.

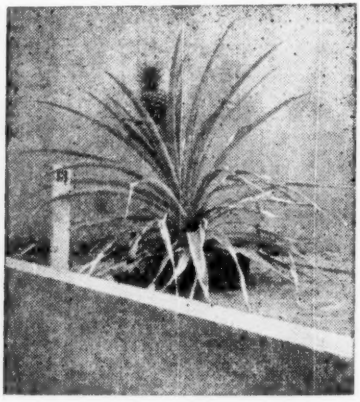


D. Treatment 11.
1 ppm of iron added.

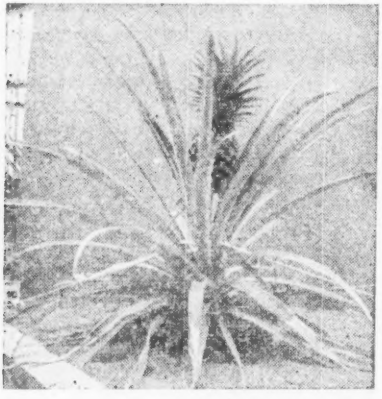
FIGURE 5.—Treatments 10 and 11, each contain: 5 ppm of manganese, 2 ppm of copper, ½ ppm of zinc, ½ ppm of boron, ½ ppm of aluminum.

Note that "C" is strongly chlorotic and necrotic, and has not flowered yet; the plant in "D" is heathly, and produced a fruit better than in "A" and "B". The plant, fruit and crown of "A" are better than those in "B". The presence of iron in "D" counteracted the detrimental action of other micronutrient elements affecting the plants in "A", "B" and "C".

PINEAPPLE PLANTS, 493 DAYS AFTER PLANTING



E. Treatment 5.
2 ppm of copper added.
The plant is necrotic. The fruit and crown are very small.



F. Treatment 9.
No copper added.
The plant is healthy. Fruit and crown are normal.

FIGURE 6.—Compare the plant in "E" with the plant in "D", figure 5. The detrimental effect of copper on plant and fruit is noticed.

2. *Root Growth*

The roots started to come out on the eighth day. On the 79 day they were rather crowded in the quart-jar vessels and were to be changed to gallon jars.

Browning or burning of the root tips was noticed in some treatments. The severity of this injury is expressed numerically in table III. This root-rot injury in pineapple roots was noticed by Schapelle (71) in cultures deprived of copper.

As is shown in table III, up to the 79 day the solution had not been aerated, and on that day the root injury due to this burning or browning of the tips was noticed in the absence of iron and copper.

In nonaerated solutions, copper showed the greatest beneficial action. Treatment 5, in which copper was added, showed almost no injury; while treatment 9, with no copper added, showed a very high severity of injury.

Aluminum showed the highest intensity of injury to roots, in the nonaerated cultures. Zinc also showed injurious effects in nonaerated solutions. Compare the results of treatments 6 and 7 with treatment 1.

The aeration of the solution, as shown on the 108 day, tended to reduce the injury in all treatments. The aluminum treatment recovered rapidly and instead of the detrimental action a highly beneficial action was noticed. In the copper treatment it was the reverse, the beneficial action of copper turned into a detrimental effect. Zinc showed recovery also.

TABLE NO. III

OBSERVATIONS ON THE ROOTS

Treatment		Total Browning of Roots		Average volume in ml.	Appearance on the 149 day
Number	Elements	unaerated 79 day	aerated 108 day		
1	ME	6	6	125	Good
2	Fe	1	0	114	Very good
3	Mn	3	3	159	Good
4	B	4	4	137	Short
5	Cu	1	5	86	Hairless, stiff & brown
6	Zn	7	4	85	Good
7	Al	10	2	173	Very good
8	ME	3	3	72	Good
9	Cu	8	1	167	Very good
	IRON MANGANESE SERIES, MIL. 5 P. P. M.				
10	Fe 0 p p m	1	0	77	Good
11	Fe 1 p p m	0	0	73	Good
12	Fe 3 p p m	0	0	101	Good
13	Fe 5 p p m	0	0	86	Good
14	Fe 10 p. p. m.	0	0	70	Good

Table 1 gives the concentrations of nutrient elements in each treatment.

Numerical evaluation of browning based on: Very slight = 1, slight = 2, medium = 3.

Volume of roots obtained by displacement of water.

The iron treatments showed beneficial action with aerated as well as non-aerated solutions. Manganese and boron showed some beneficial action.

This interesting response of the micronutrient elements, as related to the aeration of the nutrient solution, points out that as far as root growth is concerned, an oxidation-reduction process plays an important role. It seems that iron and copper have a beneficial oxidizing action under anaerobic conditions; while aluminum and zinc do not show this property. On the other hand, the action of aluminum and zinc has a beneficial effect under the conditions of ample oxygen supply in aerated solutions.

The findings of Lochwing (54), Clark and Shive (19), and Bryant (14), as to the beneficial effect of nonaerated nutrient solution on root growth, should be conditioned by saying that copper or iron are required to be present.

The beneficial action of copper in raw peat soils, pointed out by Allison, Bryan and Hunter (2), may be attributed in part to the anaerobic conditions of these soils. The detrimental effect of aluminum on root growth, as noticed by Blair (10) Barnette (6), Abbot (1), and McGeorge (57), can be explained also as due to conditions of low oxygen supply in the nutrient solution. On the other hand, the beneficial action of aluminum as observed by Schappell (71) is due to the presence of a good supply of air.

The reports of Liebig, Vanselow and Chapman, as quoted by Arnon (3), on copper toxicity in citrus roots, and the stimulating action of aluminum, are confirmed by the results shown in these experiments as to the action of copper and aluminum on root growth under aerobic conditions. That is, aluminum and copper counteract each other under either aerobic or anaerobic conditions.

The volumes of roots, as shown in table III, point out that copper and zinc tend to inhibit root growth; while aluminum, boron and manganese appear to stimulate it. Iron does not show any particular effect, for treatment 2 showed about the same results as treatment 1.

The combined effect of copper and zinc tended to produce low volume of roots in treatments 8 and 10 to 14. Treatment 9 without copper showed the best volume.

3. *Flowering and fruiting*

The first plant to flower and bear fruit was one of culture treatment 14, with 5 p.p.m. of manganese and 10 p.p.m. of iron. The fruiting stalk appeared at the 214 day, and the mature fruit was harvested on the 323 day. Table IV shows the average day when

the flowering started, and the average day when the fully mature fruit was picked from the plant in each treatment. The average weight of the fruit at maturity with and without crown and the weight of the crowns are given for each treatment. The figures on table IV show very interesting results.

TABLE NO. IV

FLOWERING AND FRUITING

MATURE FRUITS—AVERAGE FOR THREE PLANTS

Treatment		Days Taken		Weight in Grams		
Number	Elements	To Flower	To give mature fruit	Fruit	Crown	Fruit & Crown
1	ME	424	536	1,033	389	1,422
2	Fe	423	534	1,170	274	1,444
3	Mn	423	539	1,130	263	1,393
4	B	494	611	1,165	275	1,440
5	Cu	519	624	803	258	1,121
6	Zn	450	555	853	246	1,099
7	Al	431	533	1,209	338	1,547
8	ME	405	503	1,067	199	1,266
9	Cu	431	529	1,353	357	1,710
	IRON-MANGANESE SERIES—5 P. P. M. OF MANGANESE					
10	Fe 0 p. p. m.	570	675	521	160	681
11	Fe 1 p. p. m.	421	523	1,126	258	1,384
12	Fe 3 p. p. m.	412	519	1,133	319	1,452
13	Fe 5 p. p. m.	412	514	1,156	331	1,487
14	Fe 10 p. p. m.	409	509	1,230	376	1,606

See Table I for concentration of elements in each treatment.

The treatments 11 and 12 showed flowering and fruiting earlier, and the more iron, the earlier.

Copper, boron, and zinc showed a retarding influence on flowering and fruiting, as shown in treatments 4, 5, and 6 as compared with 1. Copper exerted the most detrimental effect. See figures 5 and 6. It is remarkable that no retarding action was shown by manganese in treatment 3, thus showing that it is the retarding effect of copper, boron and zinc that have been antidoted by iron in treatments 8, 9, 11, 12, 13, and 14. The retarding action in treatment 10 may be attributed to the indirect effect of manganese-chlorosis and the retarding effect of other micronutrient elements added.

The treatments with iron showed the earlier flowering and fruiting, but it appears that iron and manganese have an additive action superior to their independent action. Compare treatments 2 and 3

where these elements are alone, one in each treatment, with 8 and 11 to 14, where they are together, and against the retarding influence of copper, boron and zinc.

The weights of fruits, either with or without crown, showed to be affected quite similarly to flowering and fruiting. The treatments of copper and zinc gave the lowest yield comparing treatments 5 and 6 with 1.

It should be pointed out that the minus-copper treatment 9 showed the highest yield. The aluminum treatment also gave a high yield. Iron and boron favored high yields also.

The production of fruits was not so badly affected by the injury on roots and by chlorosis when the injury did not reach the advanced stage of destroying the plant to a certain degree, as it did in the plus-copper treatment 5 and in the no-iron treatment 10. In the iron-manganese series the weight of crop increased with the increase in iron.

The desirable vigorous crown was at its best in the manganese and iron treatments, where increasing quantities of iron were added. The treatments without zinc did not show detrimental action on the crown. It was expected that treatments without zinc might give short crowns, Hopkins et al (40), connecting it with the "little leaf" disease in citrus, or any distortion in the leaves as discussed by Hoagland (34), due to lack of zinc in the nutrient solution.

It is noticed that the aluminum treatment produced very good fruits as to weight of the fruit and of the crown. This may be due to its beneficial effect on roots when aerated.

4. *Composition of the juice of the ripe fruits.*

Table V shows the degree brix of the juice, a figure approaching the per cent of total solids by weight in solution, called also "apparent gravity solids" in sugar technology. The total sugars in juice are expressed as invert sugar. The ratio of total sugar to brix has been calculated and multiplied by 100, so as to show the relation between sugars on the total (gravity) solids, and so as to give a figure independent of dilution. This may be called the "gravity purity of invert sugars". It is the figure that should give the best criteria of the quality of the juice as to its sugar content.

The percentage acidity is reported on the basis of citric acid as discussed in the Experimental Section. The percentage of reducing sugar in the juice is also given.

TABLE NO. V

COMPOSITION OF THE JUICE OF THE RIPE FRUITS

Treatment		Juice Analysis—Average for three Plants				
Number	Elements	Reducing Sugar %	Total Sugar as Invert Sugar %	Degree Brix	o/o of solids which are Sugars	Acidity as Citric Acid %
1	ME	2.75	10.70	15.7	68.2	0.73
2	Fe	2.61	9.80	13.1	74.8	0.67
3	Mn	3.02	9.34	14.7	63.5	0.76
4	B	3.29	10.84	14.6	74.0	0.68
5	Cu	2.46	9.06	12.7	71.3	0.66
6	Zn	1.95	7.60	11.5	66.0	0.88
7	Al	2.45	8.28	11.7	70.8	0.74
8	ME	3.73	11.48	14.8	77.5	0.61
9	-Cu	2.72	9.80	13.3	73.7	0.68
	IRON-MANGANESE SERIES—5 P. P. M. OF MANGANESE					
10	Fe 0 p. p. m.	2.47	9.23	13.9	66.4	0.72
11	Fe 1 p. p. m.	4.38	9.28	12.6	73.7	0.61
12	Fe 3 p. p. m.	3.05	11.22	15.6	76.8	0.63
13	Fe 5 p. p. m.	2.66	11.35	14.8	76.8	0.64
14	Fe 10 p. p. m.	3.22	11.50	14.3	80.4	0.53

See Table I for concentrations of elements in each treatment.

The best quality of the fruits is associated with the highest ratio of sugar content to total dissolved solids, and the lowest acidity in the juice.

The sugar content of the juice was lowest in the zinc, manganese and aluminum treatments. The treatment with no micronutrient elements added showed a low sugar content. The acidity was higher in these treatments. The copper treatment showed also some detrimental action, as shown by comparing treatment 5 with copper, and treatment 9 without copper. Iron and boron showed beneficial action for increasing the sugar content and lowering the acidity. Compare treatments 2 and 4 with 1.

The iron-manganese series showed low quality of juice in treatment 10, without iron. The other treatments, 11 to 14, showed increasing content of sugar and decreasing content of acid, with the increase in iron. Iron was the most important agent of high quality. All treatments with iron were superior to treatments without iron.

The reserve micronutrient elements in the slip were not sufficient to produce good quality. The values for treatment 1 showed rather low sugar content and a high acidity. Compare treatment 1 with treatment 8, which gave the best analysis next to number 14, that had 10 p.p.m. of iron. It seems that the chlorosis-producing effect of the elements is related to low sugar content, and high acidity.

V — SUMMARY AND CONCLUSIONS

A. *Work done*

Pineapple plants were grown in nutrient solutions from uniform and healthy slips used as the propagating organ. The solutions were prepared with a mixture of macronutrient elements containing ammonia and nitrate nitrogen, potassium, phosphorus, magnesium, calcium, and sulfur. The solutions proved to be good for the growth of pineapples.

Fourteen different treatments of the micronutrient elements: iron, manganese, boron, zinc, copper and aluminum, were used in triplicate. Combinations of these elements were made in order to trace their effect, either toxic or beneficial, on pineapple plant growth and production, on root growth, on flowering and fruiting, and on the quality of the fruit. The antidoting effect of iron against the chlorosis-producing action of manganese was also studied. Plants also were grown without adding micronutrient elements to the nutrient solution.

B. *Conclusions* (See footnote on page ~~237~~ 241)

1. Iron antidotes the chlorosis-producing action of manganese in pineapple plants. With 5 parts per million of soluble manganese in a nutrient solution containing one-half part per million of boron, of zinc, and of aluminum, and 2 parts per million of copper, severe chlorosis and necrosis appeared before the pineapple plant was able to attain full growth. But, with one part per million of soluble iron humate added to a similar treatment, a healthy, normal plant was produced.

2. Iron counteracts the chlorosis-producing effect manifested by copper and boron. It raises their chlorosis-producing level.

3. Copper and manganese, at a concentration of 2 parts per million in the nutrient solution, produce strong chlorosis if iron is not present.

4. Aluminum and zinc show beneficial effects against chlorosis.

5. Iron shows no toxic effects on pineapple plants when added as the only micronutrient element to the culture solution, up to 5 parts per million. If chlorosis-producing elements like manganese or copper are present, higher concentrations are beneficial.

6. The reserve iron content in slips of the variety "Smooth Cayenne" is quite enough to counterbalance the detrimental chlorosis-producing effect of the other reserve micronutrient elements in the slip. However, plants grown in nutrient solutions deprived of all micronutrient elements produced fruits of lower sugar content than those supplied with micronutrient elements.

7. Pineapple plants respond to increase in iron in the nutrient solution, giving increase in green color, in yield, in sugar content and in decreased acid content. Treatments with a constant supply of manganese and other micronutrient elements, as explained above in (A), and with iron added in concentrations ranging from 1 to 10 parts per million, showed that the above-mentioned beneficial effect varied directly with the increase in iron added to the nutrient solution.

8. Iron prevents browning of root tips, or root rot, of pineapple plants under either anaerobic or aerobic conditions. Manganese and boron show also a somewhat beneficial action.

9. Copper has a highly beneficial action in preventing root-rot injury, when under anaerobic conditions. However, when the nutrient solution is well aerated the effect of copper is somewhat detrimental to the roots. Nonaerated solutions must be well supplied with copper and iron.

10. Aluminum has a beneficial action on root health and volume under the aerobic conditions of well aerated nutrient solutions, but it is highly toxic to the roots under anaerobic conditions.

11. Zinc exerts a somewhat detrimental action on roots under anaerobic conditions of the nutrient solution.

12. It seems that as far as root growth is concerned aeration plays an important role; copper having a beneficial action under anaerobic conditions; and aluminum when the nutrient solution is well aerated. Iron, manganese and boron are beneficial under any of the two conditions of oxygen supply.

13. Aluminum and manganese promote increased volume of roots when the nutrient solution is well aerated. Copper and zinc tend to reduce the volume of roots, but iron and boron show no direct effect.

14. Iron has a beneficial effect on early flowering and early maturity of the pineapple fruit.

15. Copper, zinc and boron, and the chlorosis-producing effect of manganese, have a retarding effect on flowering and fruiting. In

this action they are counteracted by iron. Aluminum shows no specific effect upon flowering and fruiting.

16. Zinc and copper tend to produce low yields. Aluminum and boron increase the yield. Manganese does not show specific effect on yield when added alone to the nutrient solution.

17. Zinc, copper, manganese and aluminum show a tendency to produce fruits with low sugar content and high acidity.

18. Iron and boron show a beneficial effect by increasing the sugar content and lowering the acidity. Iron is an important agent of high quality of pineapple fruits.

19. Zinc deficiency in the nutrient solution shows no signs of anatomical abnormalities in the pineapple plant.

20. Chlorosis in pineapple plants causes lower sugar content and higher ratios of acid to sugar in the fruit.

21. Experimentation on the mutual effect of the micronutrient elements on pineapple should take into consideration the reserve of elements in the planted slips.

22. The antidoting action of iron against the detrimental effects of copper should be studied by using varying concentrations of iron and different concentrations of copper.

23. Field experiments with iron humate added to the pineapple soils should be made to study its effect under soil conditions.

24. More available iron in soils will greatly improve the Puerto Rican pineapple crops.

NOTE: The above-mentioned conclusions on the independent action of the micronutrient elements refer to treatments in which they were added separately as the only micronutrient elements to the nutrient solutions in which the pineapple plants were grown. The concentrations used for each element were: 5 ppm Fe, 2 ppm Mn, 1 ppm B, 2 ppm Cu, 1 ppm Zn, and 1 ppm Al.

C. SYNOPSIS OF THE EFFECT PRODUCED BY MICRONUTRIENT ELEMENTS ON PINEAPPLE PLANTS, WHEN THE ELEMENTS ARE ADDED INDEPENDENTLY TO THE NUTRIENT SOLUTIONS

Element	PPM added to the nutrient solution	Chlorosis	Root-rot injury		Volume of roots aerated solutions	Production		
			Solutions			Early flowering and fruiting	Yields	Quality
			non-aerated	aerated				
Iron	5	Prevented Chlorosis	Prevented injury		No effect	Beneficial effect	High	High
Manganese	2	Severe Chlorosis	Beneficial effect against injury		Good	Retarding due to chlorosis	Not Specific	Low
Boron	1	Produced Chlorosis	Beneficial effect against injury		Not effect	Retarding effect	High	Good
Copper	2	Severe Chlorosis	Beneficial effect against injury	Detrimental effect	Detrimental effect	Retarding effect	Low	Low
Zinc	2	Prevented Chlorosis	Detrimental effect	No detrimental effect	Detrimental effect	Retarding effect	Low	Low
Aluminium	1	Prevented Chlorosis	Detrimental effect	Beneficial against injury	Beneficial effect	No effect	High	Low

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Unselfish encouragement of friends and wise scientific advice are, indeed, the best stimulators of the inspiration necessary to accomplish a work of this kind.

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To all, my grateful acknowledgment.

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CALCIUM BORON RELATIONSHIPS IN THE NUTRITION OF CORN AND THE DISTRIBUTION OF THESE ELEMENTS IN THE PLANTS

by Ernesto Hernández-Medina and John W. Shive

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CALCIUM-BORON RELATIONSHIPS IN THE NUTRITION OF CORN AND THE DISTRIBUTION OF THESE ELEMENTS IN THE PLANT [1]

ERNESTO HERNÁNDEZ-MEDINA [2] AND JOHN W. SHIVE [3]

The literature dealing with the inorganic nutrition of plants reveals that great attention has been given to the important role that the so-called trace elements play in the metabolic activities of different species of plants. Boron is one of these elements that has received particular attention in this regard. Both qualitative evidence and quantitative evidence (23, 28, 32, 33, 42, 43, 50) show that this element is indispensable for normal growth and development of plants and, therefore, it may be added to the previous list of essential elements (38).

Soils naturally low or deficient in boron have been reported (35, 48) in over half of the 48 states, with large areas occurring along the Atlantic coastal plain, in the Great Lakes region, and in the Pacific Northwest. If boron is not applied in usual fertilizer practices to such deficient or sub-deficient soils metabolic disturbances of the plants are likely to result in abnormal plant development as evidenced by the appearance of boron deficiency symptons and reduced crop production. Naftel (25,26,27) was one of several investigators (5,20,51) to show that liming of soils naturally low or deficient in boron is likely to cause an initiation or accentuation of boron deficiency symptoms in plants growing thereon. He found that in soils low or deficient in boron, liming decreased the water-soluble boron of the soil. On the other hand, White-Stevens (49) found that the effective level of boron in the fertilizer mixture for controlling boron deficiency in deficient soils depended upon the kind of crop grown. This suggests that care should be exercised in the application of fertilizer in soils lacking or low in boron.

The fact that it is virtually impossible (36,38) to distinguish externally between boron and calcium deficiency symptoms has sug-

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gested a possible relation between these two elements in plant nutrition. Among the first investigators to suggest a possible association between boron and calcium in the plant were Brenchley and Warington (3,41) using the broad bean as an indicator plant. They suggested that boron either enables the plant to absorb more calcium in a given period of time or permits it to be used more efficiently once it is absorbed by the plant. In the absence of boron, calcium in the roots was not utilized effectively. Later work by Marsh and Shive with corn (17) corroborated the findings of Brenchley and Warington. They found that the presence of an appropriate amount of available boron in the plant tended to maintain, in an available condition, the calcium absorbed even if no additional calcium was supplied to the plant. Evidence is also presented which demonstrates that the boron content of the culture solution did not greatly influence rates of calcium absorption by the plants. In an annual report of the New Jersey Agricultural Experiment Station the suggestion is made that boron may influence the normal metabolism of calcium in plants (29). The suggestion is sustained by the fact that no calcium was found or detected in the cell sap in the meristems of boron deficient cotton plants. Results obtained by Lowenhaupt, working with sunflower (16), with reference to calcium content of tissues are in agreement with the view that there is a relationship between the boron available to the plant and the utilization of calcium. Haas (11) working with citrus and walnuts found no evidence in the leaves of any relationship between the soluble boron and the soluble calcium in the same tissues; while Muhr (24) working with sugar beets, radishes, chickory, turnip, barley, wheat, dandelions, mangels, rutabaga and corn, and Hill and Grant (12) with turnips found that tissue of plants inadequately supplied with boron contained, in general, a higher percentage of calcium than the tissue grown with sufficient boron. Still, there is a diversity of opinion among investigators as to the determinative role of boron in the accumulation of calcium in the plant. Haas (10) working with citrus found that the percentage of total calcium that was water soluble was higher in leaves of plants which received large quantities of boron in the substrate than in the leaves of plants receiving lower levels of boron in the nutrient medium. On the other hand, Holley and Dulin (13), Morris (22), and Dmitriev (7) furnished evidence to demonstrate that the presence or absence of boron in the nutrient media had no influence on the quantity of calcium in the plant tissue analyzed. As has been indicated (31) some disagreement may be due to the use of different species of plants, dif-

ferent growing media (sand and water cultures, in some cases, soil, in others) varying climatic conditions, and differences in the boron concentrations involved.

Later work (4,6,14) has furnished evidence that a definite balance exists between the calcium and boron content of healthy tomato, tobacco and oat plants. Brennan (4) has indicated that normal tomato plants have an intermediate numerical ratio of total calcium to total boron whereas boron deficient plants and boron toxic plants have abnormally high and abnormally low ratios, respectively.

The purpose of this investigation was to study further the calcium-boron relationship in the corn plant and to determine the distribution of these elements in this monocotyledon.

SERIES I

Methods

Cultural methods—This experiment was the first of two series and was started on October 16, 1945. The plants were grown in sand culture. Corn seeds of Rutgers Hybrid No. 2 were used. They were selected for uniformity in shape and size and were sowed directly in purified sand in 9 inch highly glazed pots. The sand was previously washed with tap water, then treated with 2 per cent sodium hydroxide and left overnight. Following this, it was flushed with distilled water, treated with 2 per cent hydrochloric acid and again left overnight, after which it was completely flushed with distilled water until free from chloride ions as determined by the silver nitrate test. This sand treatment was necessary to remove any materials which might furnish boron and also to free the sand from other nutrients. Four seeds were planted in each pot and the sand was flushed with a dilute nutrient solution of the following composition: KH_2PO_4, 0.0016 M.; $NaNO_3$, 0.0038 M.; $MgSO_4$, 0.0028 M.; $CaCl_2$, 0.0025 M. with the usual traces of iron, zinc and manganese but with no boron. Four days after germination the best three seedlings were left for treatment. The rejected seedling was removed by first flushing the sand with distilled water and then gently pulling it out with its entire root system. The cultures were maintained on the diluted culture solution for a week to develop uniform plants before nutrient treatments were started. At the end of this period all plants appeared vigorous and fairly uniform and the calcium-boron nutrient treatments were begun and maintained for 64 days, using the continuous solution renewal method of Shive and Stahl (39,40). Approximately two liters of nutrient solution per day were used for each culture containing three plants.

In this experiment there was a total of forty-eight cultures, which were divided into six groups of eight cultures each. Each group was grown at a different level of calcium, as follows: 5.0, 10.0, 50.0, 100.0, 250.0, and 500.0 p.p.m. Each of the eight cultures in a given calcium level received boron in eight different concentrations. The levels of boron used were as follows: 0.0, 0.001, 0.01, 0.10, 0.25, 5.0, 10.0, and 20.0 p.p.m. Figure 1 ilustrates the plan of the experiment the number on each block representing culture numbers. The composition of the culture solutions with the different levels of calcium used with each of the eight boron levels is presented in table 1. Salts of analytical grade were used without further repurification. Boron was supplied in the form of borie acid. The ammonium sulfate was used to aid in the prevention of iron-deficiency chlorosis.

At the end of the experimental period, on December 28, when definite symptoms of boron deficieney and toxicity occurred on plants receiving deficient and very high nutrient levels of boron respectively, the plants on the different treatments were harvested. After green and dry weights of the tops had been obtained, the plants were cut with a stainless steel knife into small pieces which were thoroughly mixed so that representative samples could be obtained for analysis.

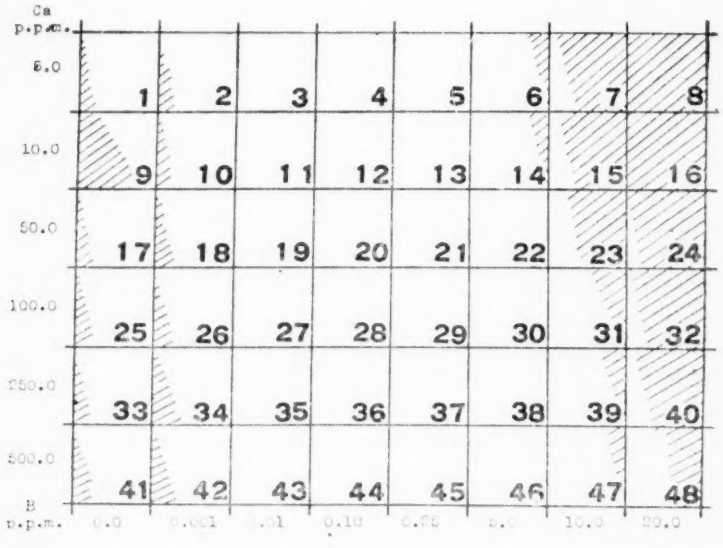

FIG. 1.—Diagram showing calcium and boron treatments and general experimental plan. Relative proportion of shaded area of each block at the left denotes relative degree of boron deficiency symptoms, those at the right boron toxicity symptoms.

TABLE NO. 1

COMPOSITION OF CULTURE SOLUTIONS AT EACH OF THE 6 DIFFERENT NUTRIENT LEVELS OF CALCIUM USED WITH EACH OF 8 DIFFERENT BORON CONCENTRATIONS OF 0.0, 0.00', 0.01, 0.10, 0.25, 5.0, 10.0, AND 20.0 P. P. M.*

Culture Numbers	Calcium Levels	Molar Concentration of Salts						
		KH_2PO_4	K_2SO_4	$NaNO_3$	$MgSO_4$	$CaCl_2$	$CaSO_4$	$(NH_4)_2SO_4$
	p. p. m.							
1–8	5	.00113	.00113	.0085	.00113	.000125	.....	.0004
9–16	10	.00113	.00113	.0085	.00113	.00025	.....	.0004
17–24	50	.00113	.00113	.0085	.00113	.00125	.....	.0004
25–32	100	.00113	.00113	.0085	.00113	.0025	.....	.0004
33–40	250	.00113	.00113	.0085	.00113	.00625	.....	.0004
41–48	500	.00113	.00113	.0085	.00113	.00625	.00625	.0004

* TRACE ELEMENTS:- Each nutrient solution contained 1 p. p. m. iron, 0.5 p .p. m. zinc, and 0.25 p. p. m. manganese used in the form of sulfates.

In order to determine the soluble boron and calcium in the plant tissue, a 50–gram sample of fresh tissue from each culture was frozen in a refrigerator immediately at time of harvest and kept at approximately minus 18°C until ready for analysis. In addition, 100–gram samples of fresh tissue were weighed and dried as described below to be used in the determinations for total boron and total calcium in each aliquot.

At the time of harvest one of the three plants from a culture receiving a high and a low boron level in each calcium series was harvested separately and fractionated into top leaves, middle stem and lower stem in order to make a preliminary study of the distribution of the total boron and calcium in these portions of the plant. The fraction designated as "top leaves" consisted of the leaves extending above and including the apical meristem; the "middle stem" fraction consisted of the culm tissue including leaf sheath but without blades extending from the base of the apical meristem to the node second removed from the sand surface; the "lower stem" fraction consisted of the lower two nodes and internodes of the culm including leaf sheaths but without blade tissue.

Chemical Methods—Dry weights of the 100–gram samples of green tissue taken for analysis at the time of harvest were obtained after oven drying at 70°C for 48 hours. The dried tissue was then ground in a semi-micro Wiley mill to pass a 40 mesh screen, after which 2–gram samples were weighed into porcelain crucibles and ignited in a muffle furnace starting at room temperature and gradually raising the temperature to 600°C. The samples were held at this temperature for approximately 6 hours which resulted in complete ashing. The ash was dissolved in 20 ml. of (1 +4) HCl, transferred

to a 100 ml. volumetric flask and made to volume. Aliquots of this solution were then taken for determination of total boron and total calcium.

For the determination of tissue contents of soluble boron and soluble calcium, the frozen 50–gram tissue samples were thawed and then placed in a piece of muslin of suitable size fitted in a nickel plated steel cylinder 5.71 centimeters inside diameter, covered with 40 ml. of distilled water, and subjected to a pressure of 2500 pounds per square inch for one minute in a Carver Press. The press cake was washed twice more with 40 ml. of distilled water and subjected to the same pressure but after the last washing the pressure was maintained for two minutes. The extracted plant juices and washings were passed through a quantitative Whatman number 5 filter paper using suction. The press cake was then removed from the muslin and together with the filter paper was oven-dried at 70°C for 48 hours. After drying, press cake and filter paper were ignited in a muffle furnace and the ash was dissolved and the solution made to volume as previously described. Aliquots of these samples were taken for the determinations of boron and calcium, respectively. Soluble boron and soluble calcium contents of the tissue were determined by the differences between the analytical values of these two elements in the unextracted sample and the extracted press cake.

In this work the soluble portions of boron and of calcium are considered to be those which were extracted from the tissue samples under pressure by the method described and these soluble portions are also considered to be the active portions in the tissue at least at the time of harvest. Those portions of the calcium and boron which remain in the plant tissue in the press cake after extraction of the juices by the above method are considered to be the insoluble and inactive portions.

The official micro-method of the Association of Official Agricultural Chemists (1) with a few minor changes was used for the calcium determinations. Since it is not necessary to remove the silicon dioxide, this step was omitted (21). Coprecipitation of the silicon dioxide with calcium oxalate does not interfere in the titration of the oxalate ions by the permanganate ions. To obtain greater accuracy in the results 0.01 N $KMnO_4$ was used for titrating the oxalate ion instead of 0.02 N solution recommended in the official method.

The Berger and Truog (2) colorimetric method was used for the quantitative determination of boron with modifications similar to those of Marsh and Shive (17) except that 10 ml. quantities of sul-

furic acid-quinalizarin reagent were used with 1 ml. of the test solutions instead of the 50 ml. quantities used by Marsh and Shive. The 50 ml Nessler tubes used for color comparison of standards and test solutions were kept tightly closed during the determinations. Sulfuric acid, 95.5 per cent by weight, was used in this method instead of the 98 per cent acid employed by Berger and Truog.

Results

Character of Plants after Treatment.—The relative intensities of the visible symptoms of metabolic disorder resulting respectively from deficient and excess nutrient concentrations of boron in the culture solution are represented by the shaded areas in figure 1. All the plants at the two lowest boron levels, 0.00 and 0.001 p.p.m. exhibited boron deficiency symptoms. However, as is indicated in figure 1, none of these boron deficient plants were very seriously injured and, moreover, no definite relation is observable between severity of boron deficient symptoms and relative calcium concentrations of the substrate. The injury due to boron deficiency which occurred in boron deficient cultures in this experiment ranged from slight to medium intensity and was not so severe as that in similar nutrient treatments in the experiment to be reported later in this paper. The boron deficiency symptoms were characterized by the appearance of elongated white transparent interveinal stripes on the newly formed leaves (44) and were similar but not quite so severe as those on leaves of plants shown in figure 2, which were grown in a subsequent experiment. The roots of the boron deficient plants were slightly brown, and the root systems were not so extensive as those of plants receiving boron. These symptoms were especially severe at the low calcium levels probably as a result of both boron and calcium deficiencies.

It has been reported (46) that under the short day conditions of spring and autumn the onset of boron deficiency symptoms is delayed as compared with long day conditions of summer. Since this experiment was run during the fall season it is possible that the short length of day may account for the fact that the boron deficiency symptoms were not so severe as those which developed in boron deficient cultures grown in the subsequent experiment conducted under the long day conditions of late spring. No distortion or injury was observed in apical meristems of boron deficient plants.

Figure 1 shows that slight boron toxicity symptoms appeared in cultures number 6 and number 14 receiving 5.0 p.p.m. boron at the two lowest calcium levels. Plants in other cultures grown at this boron level exhibited no toxicity symptoms. At the boron concentrations

of 10.0 p.p.m., boron toxicity symptoms were more severe than at 5.0 p.p.m. and appeared to some extent in plants at all calcium levels. At the boron level of 20.0 p.p.m. the metabolic disturbances due to the high boron concentration were most pronounced and were especially severe at the three lowest calcium levels.

The plants grown at 20.0 p.p.m. boron and with 5.0 p.p.m. of calcium (figure 3) showed more serious symptoms of boron toxicity than the plants grown at the same boron level but with 500.0 p.p.m. calcium (figure 4). Boron toxicity symptoms consisted of stunted

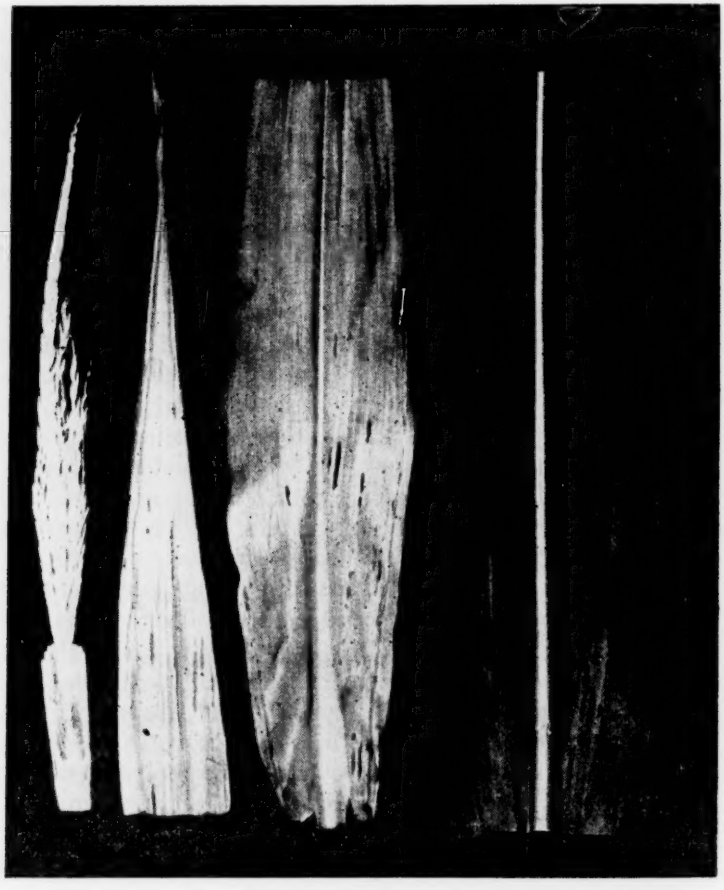

FIG. 2.—Typical boron deficiency symptoms of growing point and top leaves of corn plant grown at nutrient levels of 250.0 p.p.m. calcium and 0.001 p.p.m. boron.

growth, pale yellow-green color of the youngest leaves with yellow colored, dying and dead marginal tissue appearing largely on the older leaves. Typical boron toxicity symptoms are shown in figure 5 on the leaves of plants grown in a subsequent experiment. The root system of boron toxic plants were relatively small and the roots were brown in color. The appearance of these root systems was similar to those of plants receiving similar treatments and grown in a subsequent experiment.

Calcium deficiency symptoms were observed to some extent in the younger leaves of plants receiving 5.0 and 10.0 p.p.m. calcium. These symptoms consisted of distortions, breaks, and colorless areas

FIG. 3.—Corn plants grown in culture number 8 at nutrient levels of 5.0 p.p.m. calcium and 20.0 p.p.m. boron.

of tissue on the margins and tips of the leaves. These calcium deficiency symptoms are similar to, but not so serious as those which occurred on leaves of plants grown in a subsequent experiment. (Figure 6).

With regard to green and dry weights of the whole tops of the plants grown with the various calcium-boron nutrient levels it was found that with few exceptions green and dry weights increased as the calcium concentration of the substrate increased at any given boron level. The green and dry weights of the plants receiving the highest nutrient levels of boron 5.0, 10.0 and 20.0 p.p.m. within

FIG. 4.—Corn plants grown in culture number 48 at nutrient levels of 500.0 p.p.m. calcium and 20.0 p.p.m. boron showing mild symptoms of boron toxicity.

a given calcium level were considerably lower than the weights of those plants receiving lower nutrient levels of boron.

Results of Chemical Analyses.—The results of quantitative analyses for boron and calcium contents of the tissues, both total and soluble, are presented in table 2. It appears from the analytical data that both total boron and soluble boron at any given nutrient level of boron are more or less independent of the calcium concentration in

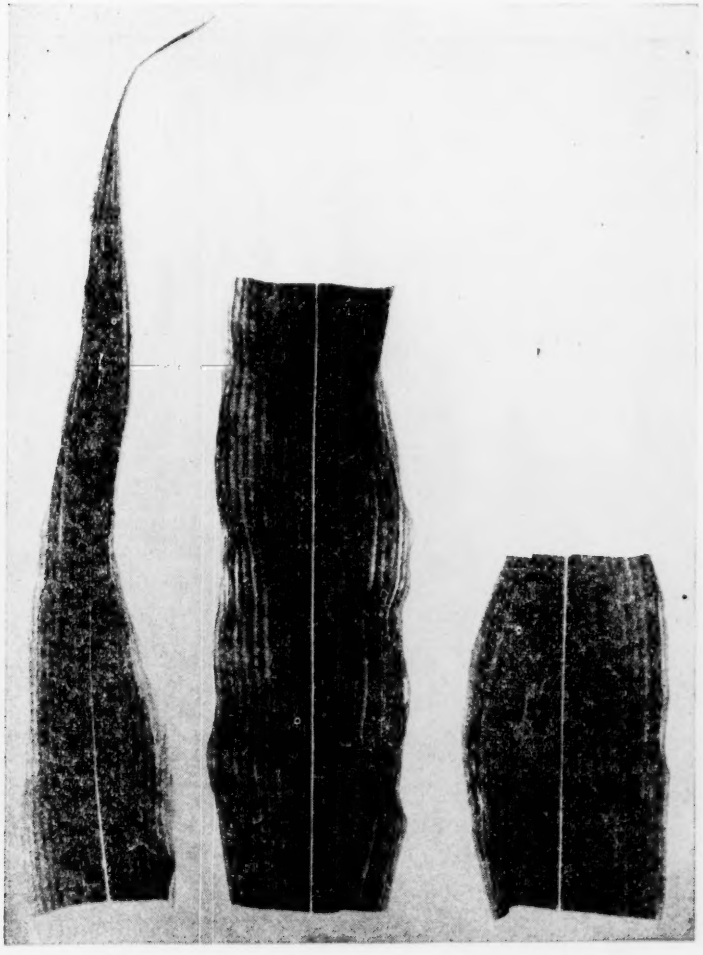

FIG. 5.—Typical boron toxicity symptoms of lower leaves of corn plant grown at nutrient levels of 100.0 p.p.m. calcium and 20.0 p.p.m. boron.

the substrate. However, the plants receiving highest nutrient concentrations of boron (20.0 p.p.m.) contained somewhat lower tissue contents of boron, both total and soluble, when grown at the highest calcium level (500.0 p.p.m.) than plants grown at equivalent boron level but at lower calcium concentrations. This suggests that boron accumulation by the plant was modified in some way at the highest calcium level. These results support the qualitative observations wherein boron toxicity symptoms of plants receiving the highest

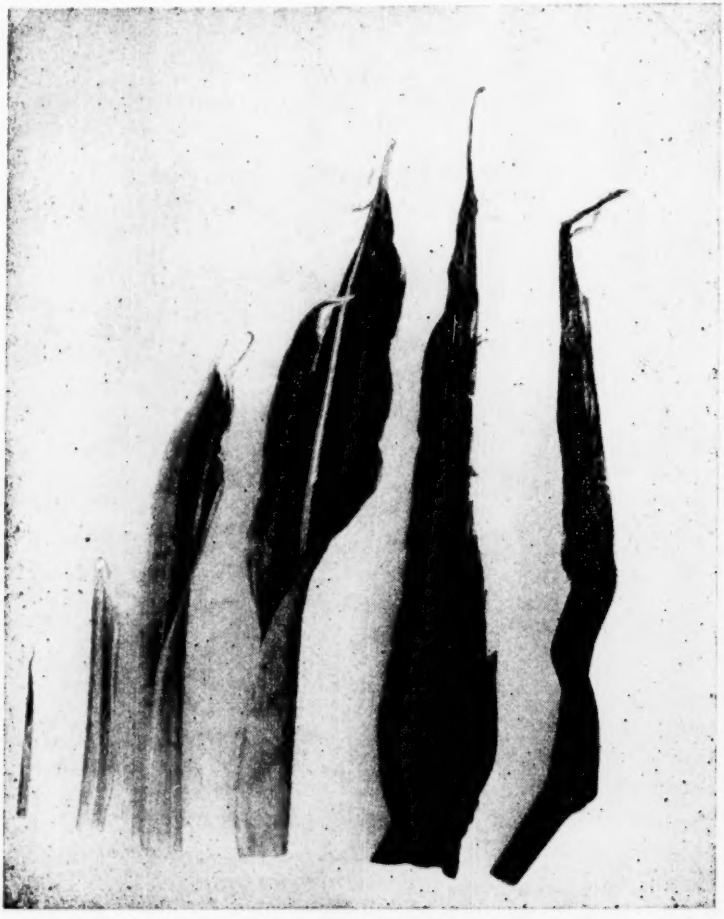

FIG. 6.—Leaves showing well defined calcium deficiency symptoms of corn plant grown at nutrient levels of 5.0 p.p.m. calcium and 0.25 p.p.m. boron.

TABLE NO. 2

TOTAL AND SOLUBLE CALCIUM AND BORON IN MILLIGRAMS PER GRAM DRY TISSUE OF WHOLE TOPS OF CORN PLANTS OF SERIES I

Treatment		Total	Soluble	Soluble B Per cent of Total	Total	Soluble
Ca	B	B	B		Ca	Ca
p. p. m.	p. p. m.	mgm. p. g.	mgm. p. g.	Per cent	mgm. p. g.	mgm. p. g.
5	0.0	0.010	0.002	20.0	0.84	0.53
5	0.001	0.015	0.007	46.6	1.08	0.56
5	0.01	0.020	0.008	40.0	0.95	0.63
5	0.10	0.023	0.012	52.1	1.14	0.77
5	0.25	0.030	0.016	53.3	1.36	1.00
5	5.0	0.300	0.206	68.8	1.00	0.51
5	10.0	0.450	0.388	86.2	1.18	0.85
5	20.0	0.750	0.567	74.6	0.91	0.54
10	0.0	0.010	0.002	20.0	1.63	1.10
10	0.001	0.015	0.006	40.0	1.41	0.84
10	0.01	0.020	0.009	45.0	1.25	0.71
10	0.10	0.023	0.015	65.2	1.51	1.06
10	0.25	0.030	0.016	53.3	1.94	1.24
10	5.0	0.174	0.059	33.9	1.57	1.17
10	10.0	0.424	0.266	62.7	1.65	1.13
10	20.0	0.997	0.610	61.1	1.58	1.02
50	0.0	0.020	0.008	40.0	3.69	2.12
50	0.001	0.020	0.009	45.0	3.11	1.97
50	0.01	0.025	0.013	52.0	3.10	2.02
50	0.10	0.030	0.017	56.6	4.00	2.30
50	0.25	0.031	0.019	61.2	3.09	1.27
50	5.0	0.175	0.119	68.0	3.53	2.61
50	10.0	0.424	0.358	84.4	3.75	2.09
50	20.0	0.765	0.557	72.2	4.59	2.76
100	0.0	0.015	0.008	53.3	5.18	3.22
100	0.001	0.015	0.009	60.0	4.93	2.34
100	0.01	0.025	0.015	60.0	4.75	2.75
100	0.10	0.032	0.019	59.3	4.78	2.88
100	0.25	0.040	0.024	60.0	4.72	3.93
100	5.0	0.250	0.204	80.0	5.62	3.75
100	10.0	0.449	0.384	85.5	4.65	3.25
100	20.0	0.750	0.574	76.5	6.72	4.25
250	0.0	0.015	0.008	53.3	5.80	4.11
250	0.001	0.015	0.007	46.6	6.78	5.40
250	0.01	0.025	0.015	60.0	6.08	4.50
250	0.10	0.035	0.021	60.0	5.93	4.47
250	0.25	0.035	0.018	51.6	5.73	4.13
250	5.0	0.137	0.087	63.5	5.93	4.46
250	10.0	0.350	0.289	82.5	5.73	4.34
250	20.0	0.750	0.531	70.8	8.05	6.01
500	0.0	0.015	0.007	46.6	6.64	5.14
500	0.001	0.015	0.007	46.6	6.70	5.50
500	0.01	0.020	0.012	66.0	6.61	4.95
500	0.10	0.030	0.015	50.0	7.37	5.77
500	0.25	0.030	0.021	70.0	6.33	5.07
500	5.0	0.128	0.093	72.6	7.12	5.74
500	10.0	0.424	0.241	56.8	6.60	5.07
500	20.0	0.624	0.428	68.6	7.98	6.26

nutrient level of boron (20.0 p.p.m.) were less severe at the highest calcium level (500.0 p.p.m.) than at lower calcium levels. These differences in severity of boron toxicity symptoms were also reflected in the green and dry weight yields of the plants. Parks et al working with tomatoes obtained reduced green and dry weights of plants receiving toxic quantities of boron (31).

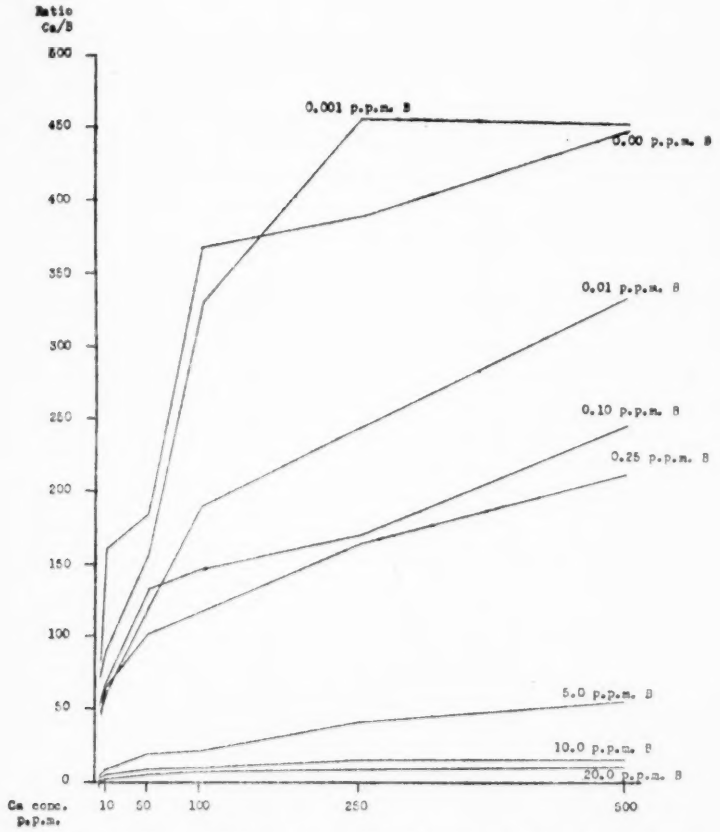

FIG. 7.—Ratios of total calcium to total boron of whole tops of corn plants grown at different boron levels plotted against calcium concentration in the substrate.

The experimental results also show that, in general, at each nutrient level of calcium a relatively higher percentage of the total boron remains soluble in the corn plant at the three highest boron levels than at the lower boron concentrations. This is similar to results obtained by Marsh with corn in which the lowest and highest percentage of soluble boron occurred at deficient and toxic boron substrate levels respectively (18).

Within any given calcium level the tissue contents of soluble boron were smaller in the plants grown at the two lowest boron levels than at the higher boron levels (table 2). Plants containing the lowest soluble boron at any given calcium level showed evidence of typical boron deficiency symptoms. This suggests that in this ex-

TABLE No. 3

RATIOS OF DRY WEIGHT TISSUE CONTENTS OF TOTAL CALCIUM TO TOTAL BORON OF WHOLE TOPS OF CORN PLANTS OF SERIES I

Treatment		Total Ca / Total B	Treatment		Total Ca / Total B
Ca	B		Ca	B	
p. p. m.	p. p. m.		p. p.m.	p. p. m.	
5	0.0	84	100	0.0	368
5	0.001	72	100	0.001	331
5	0.01	48	100	0.01	191
5	0.10	51	100	0.10	148
5	0.25	45	100	0.25	118
5	5.0	3	100	5.0	22
5	10.0	25	100	10.0	10
5	20.0	1	100	20.0	9
10	0.0	163	250	0.0	389
10	0.001	92	250	0.001	455
10	0.01	63	250	0.01	244
10	0.10	67	250	0.10	170
10	0.25	65	250	0.25	164
10	5.0	9	250	5.0	43
10	10.0	4	250	10.0	16
10	20.0	1.6	250	20.0	11
50	0.0	185	500	0.0	446
50	0.001	156	500	0.001	449
50	0.01	120	500	0.01	323
50	0.10	133	500	0.10	246
50	0.25	104	500	0.25	217
50	5.0	20	500	5.0	56
50	10.0	9	500	10.0	16
50	20.0	6	500	20.0	13

periment a constant nutrient supply of boron ranging from 0.01 to 0.25 p.p.m. was required to allow corn plants to grow normally.

From the results with regard to total and soluble calcium presented in table 2, it is evident that total calcium content of the tissue is mainly determined by the calcium concentration in the substrate and is largely independent of the nutrient concentration of boron. At each of the three highest levels of calcium, plants grown with the highest boron treatment had a considerably higher total and soluble calcium content than plants grown with the other boron treatments. These differences appear large enough to have some significance but their determinative factors are not clear.

From the analytical data relating to calcium and boron contents of the plant, the ratios of total calcium to total boron for the plants grown at the different calcium and boron levels have been calculated and presented in table 3. In figure 7 are presented the same Ca/B ratios plotted against calcium concentrations of the substrate. It is evident from the ratios of table 3 that the tissue of boron deficient plants receiving nutrient treatments of 0.0 and 0.001 p.p.m. boron had the highest Ca/B ratios at any given nutrient level of calcium while the Ca/B ratios of the tissues of boron-toxic plants were the lowest. Tissues of normal plants receiving intermediate amounts of

boron had intermediate ratios. It appears from the results that the quantitative relationship between calcium and boron in the tissues greatly influences the metabolic activities of the corn plant. The qualitative observations made on the plants (figure 3 and 4) which exhibited symptoms of boron toxicity seem to be definitely associated with the calcium and boron contents of these plants. At 500.0 p.p.m. calcium and 20.0 p.p.m. boron, the boron toxicity symptons were considerably less severe than those of plants grown with the same nutrient level of boron but at lower calcium levels. They also had the highest Ca/B ratio, 13 to 1, while the plants grown at 5.0 p.p.m. calcium and 20.0 p.p.m. boron had a Ca/B ratio of only 1 to 1 (table 3.). Although the proportion of calcium to boron in the plants in the first case mentioned above (500.0 p.p.m., 20.0 p.p.m. boron) was probably not the optimum for normal plant metabolism, nevertheless it seemed to be associated in some way with less severe boron toxicity than was evident in the plants grown at the highest boron level at the lowest calcium concentration. These were the plants that had a very low Ca/B ratio (figure 3). It can also be seen from table 3 that as the boron increased at a given calcium level the Ca/B ratio decreased.

Results of Chemical Analyses of Tissue Fractions of Corn Plant Tops

The results of quantitative tests for total boron and total calcium of the tissue fractions of plants grown at low and high boron levels

TABLE NO. 4

TOTAL BORON AND TOTAL CALCIUM OF TOP LEAVES, MIDDLE STEM, AND LOWER STEM OF CORN PLANTS OF SERIES I EXPRESSED AS MILLIGRAMS PER GRAM DRY TISSUE

Treatment		Top Leaves		Middle Stem		Low Stem	
Ca	B	Total B	Total Ca	Total B	Total Ca	Total B	Total Ca
p. p. m.	p. p. m.	mgm. p g.	mgm. p g.	mgm. p g.	mgm. p g.	mgm. p g.	mgm. p g
5	0.001	0.025	0.63	0.020	1.43	0.020	0.65
5	5.0	0.180	0.43	0.325	0.92	0.080	0.58
10	0.01	0.025	1.10	0.025	1.98	0.020	0.94
10	5.0	0.175	0.89	0.275	1.76	0.080	0.82
50	0.01	0.030	2.19	0.025	5.02	0.020	2.41
50	5.0	0.175	2.83	0.275	3.52	0.100	1.65
100	0.10	0.035	3.04	0.035	4.97	0.030	2.89
100	5.0	0.199	2.70	0.225	5.19	0.080	3.94
250	0.01	0.030	5.39	0.030	8.46	0.010	6.18
250	5.0	0.325	5.16	0.250	7.31	0.070	5.94
500	0.001	0.030	4.23	0.020	6.41	0.010	6.28
500	5.0	0.275	4.39	0.300	10.16	0.080	8.25

(0.001, 0.01, 0.10 and 5.0 p.p.m.) at each of the six calcium concentrations are presented in table 4. These were the plants which were divided into top leaves, middle and lower stem fractions. As was pointed out before, this preliminary study was carried out to obtain an idea as to the distribution of the total calcium and boron in these three different portions of the corn plant. An analysis of the data shows that in general at the lowest boron level and at each nutrient level of calcium, boron is more or less equally distributed among top leaves, middle stem, and lower stem, with the least amount found in the lower stem tissue. However, at the highest boron level, and at each calcium level, the boron content of the middle stem was greater than that of the top leaves with but one exception, and the lower stems had the least amount of boron at this boron level.

It is also evident from the results presented in table 4 that the tissue content of calcium was greater in the middle stem portion of the plant at any given nutrient level of calcium. The amount of calcium found in each tissue was directly related to the calcium concentration in the substrate, with one exception, and was not greatly influenced by the boron concentration of the nutrient solution, with two exceptions. This occurred in the case of plants grown with the culture solution containing both 5.0 p.p.m. boron and 500.0 p.p.m. calcium where the calcium content of the middle and lower stem was considerably more than the calcium content of the respective tissue fractions of plants grown at 500.0 p.p.m. calcium but with only 0.001 p.p.m. boron.

Table 4 shows also that a relatively large amount of calcium accumulated in the lower stem of the plant at any given calcium level. But at the highest calcium levels (250.0, 500.0 p.p.m.) the lower stem had considerably greater tissue content of calcium than the top leaves. Thus it is evident that at these two highest calcium concentrations a large proportion of calcium accumulated in the lower or older part of the plant and was not freely translocated to the upper or younger leaves of the plant.

SERIES II

Methods

Cultural Methods.—The second corn series was started on April 18, 1946 to study the response of plants under different environmental conditions from those under which the first experiment was conducted. As before, seeds of Rutgers Hybrid No. 2 field corn selected for uniform size and shape were used in this test. Five

seeds were planted in each pot, and after germination the best three seedlings were left to receive treatments. During the first week of growth all corn cultures were supplied with the same dilute culture solution used during the first week with Series I. No boron was added during this period in order that the seedlings might utilize the supply of this element in the seed as far as possible before treatments were started. During the second week of growth all cultures received the same nutrient solution except that boron was added at the rate of 0.01 p.p.m. At the end of the second week all cultures were vigorous and appeared healthy in all respects.

The calcium-boron nutrient treatments were started beginning with the third week and were maintained for 30 days. Before applying treatments to plants, the sand in each culture was flushed with distilled water, then flushed again with the nutrient solutions to be used according to the experimental plan. As before, nutrient solutions were applied by the continuous solution renewal method. The culture solutions used with the different nutrient levels of calcium and of boron as well as the experimental setup were the same as in Series I. The calcium chloride salt used in the culture solutions of this series was purified by recrystallization after it was found by analysis to contain small amounts of boron. At the end of the experimental period of 30 days, plants were harvested. As before, green and dry weights of the tops were obtained and frozen samples of the plants of each culture were prepared for analysis. In addition, one of the three plants from a culture in each of the six different calcium levels and receiving 0.0, 0.25, and 20.0 p.p.m. boron respectively, was divided into four fractions—top leaves, top stems, lower leaves, and lower stems—for determinations of total and soluble boron and calcium of each tissue.

In the above tissue fractions the "top leaves" consisted of the apical meristem and the leaves arising in its immediate proximity and extending above it. The "top stems" consisted of the culm tissue starting downward from the base of the apical meristem and including the uppermost two differentiated internodes without attached blades and sheaths. The "lower leaves" consisted of all the leaves of the stem below the level of the apical meristem. The "lower stem" fraction consisted of the nodes and internodes below the base of the "top stems", as defined above.

Chemical Methods.—The same procedure and methods used in the first series were followed for the determination of total and soluble boron and calcium in the tissues of this corn series. Because

of the lack of sufficient plant material in some of the tissue fractions it was necessary in those cases to ignite a 1-gram instead of a 2-gram sample and to make to 50 ml. instead of 100 ml. volume.

Results

Character of Plants after Treatments.—The relative intensities of the external symptoms of metabolic disturbance due to deficient and toxic quantities of boron in the nutrient media are represented by the proportion of the shaded areas in the blocks of the diagram of figure 8. If this diagram is compared with figure 1 a striking difference will be noted in the response of the plants to the low and high boron treatments at the various calcium concentrations. Symptoms of boron deficiency appeared first in the plants at the highest nutrient calcium concentrations, 19 days after treatment. At the end of the experimental period all plants at the two lowest boron levels of 0.0 and 0.001 p.p.m. showed boron deficiency symptoms, except those grown at the two lowest calcium levels. The boron deficiency symptoms increased in severity progressively as the calcium concentration of the substrate increased, except that somewhat more intense boron deficiency symptoms were detected at 250.0 p.p.m. calcium than at 500.0 p.p.m. calcium concentration.

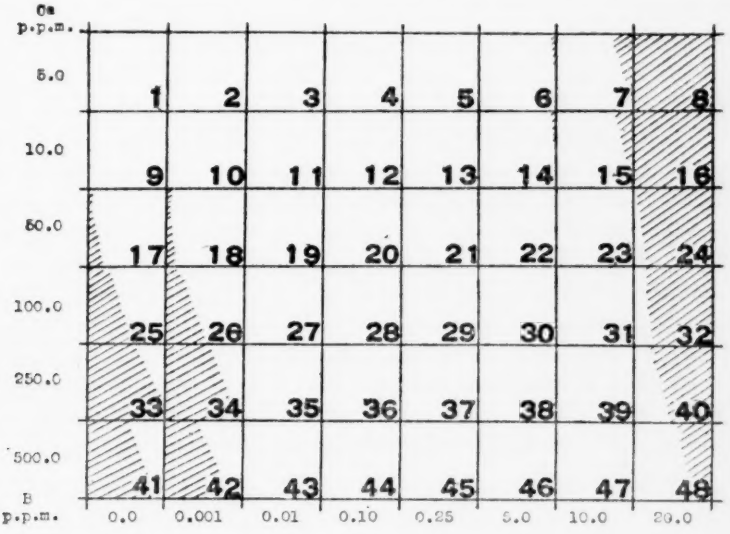

FIG. 8.—Diagram showing calcium and boron treatments and general experimental setup of Series II. Proportionate amounts of shading in the blocks indicate approximate relative severity of boron deficiency symptoms (left) and boron toxicity symptoms (right).

The growing points of boron deficient plants showed a brownish color as compared to the creamy-white color of growing points of healthy plants. The roots were brown and the root systems were less extensive than those of plants receiving adequate amounts of boron. As before, the boron deficiency symptoms of the roots were most severe at the low calcium levels. This was probably due to combination of both boron and calcium deficiency.

In this series, in contrast to the first series (figures 1, 8), there was virtually no indication of boron toxicity at 5.0 p.p.m. boron. With the exception of the plants at the two calcium levels of 5.0 and 10.0 p.p.m., where boron toxicity was only slight, the plants at other calcium levels did not show evidence of boron toxicity at 10.0 p.p.m. boron. However, as is evident from figure 8, injury due to boron toxicity occurred at 20.0 p.p.m. boron at all calcium levels but decreased in severity with increasing calcium concentration in the substrate. As in Series I, boron-toxic plants were characterized by a yellow-green color of the top leaves and by brown, dead margins and tips of the older or lower leaves. The roots of these plants were mostly brown and less abundant, especially at the two lowest calcium levels, as compared with the long, silvery-white and healthy roots of the plants receiving boron ranging between 0.01 to 0.25 p.p.m. Roots of plants receiving 5.0 and 10.0 p.p.m. boron showed slight boron injury as evidenced by their light brown color as compared with the silvery-white color of roots of plants supplied with lower concentrations of boron.

In the plants of this series the severity of calcium deficiency symptoms was considerably more pronounced in plants grown at 5.0 and 10.0 p.p.m. calcium than in the same treatments in Series I planted in the fall season. Plants were most severely injured at the lowest calcium level (5.0 p.p.m.) and were stunted considerably in vegetative growth (30). In some of the plants the young leaves failed to unroll properly and the tips of these cylinders of unrolled leaf tissue were dead or dying. The root systems of these calcium deficient plants were less extensive and were slightly browner than the roots of plants receiving larger amounts of calcium in the substrate. This probably indicates an early maturity of the tissues.

Results of Chemical Analyses.—The results of quantitative tests for total and soluble boron and calcium are presented in table 5. An examination of these data clearly demonstrates that the total and soluble boron in the corn plant is largely a direct function of the boron concentration in the substrate and is only slightly in-

TABLE No. 5

TOTAL AND SOLUBLE CALCIUM AND BORON IN MILLIGRAMS PER GRAM DRY TISSUE OF WHOLE TOPS OF CORN PLANTS OF SERIES II

Treatment		Total	Soluble	Soluble B Per cent of Total	Total	Soluble
Ca	B	B	B		Ca	Ca
p. p. m.	p. p. m.	mgm. p. g.	mgm. p. g.	Per cent	mgm. p. g.	mgm. p. g.
5	0.0	0.020	0.007	30.0	0.61	0.30
5	0.001	0.030	0.013	43.3	0.72	0.30
5	0.01	0.040	0.022	55.0	0.61	0.31
5	0.10	0.045	0.026	57.7	0.78	0.39
5	0.25	0.045	0.025	55.5	0.66	0.25
5	5.0	0.175	0.132	75.3	0.63	0.31
5	10.0	0.424	0.358	84.7	0.69	0.22
5	20.0	0.624	0.529	84.7	0.54	0.18
10	0.0	0.020	0.006	20.0	0.97	0.55
10	0.001	0.025	0.013	52.0	0.81	0.51
10	0.01	0.030	0.015	33.3	0.86	0.53
10	0.10	0.035	0.023	65.7	0.92	0.58
10	0.25	0.035	0.025	71.4	0.92	0.42
10	5.0	0.175	0.147	84.0	0.78	0.39
10	10.0	0.325	0.265	81.5	0.88	0.54
10	20.0	0.625	0.536	85.7	0.77	0.45
50	0.0	0.025	0.011	44.4	2.87	1.96
50	0.001	0.025	0.013	52.0	2.33	1.62
50	0.01	0.030	0.017	56.6	2.64	1.72
50	0.10	0.035	0.023	65.7	2.58	1.83
50	0.25	0.040	0.025	62.5	2.11	1.24
50	5.0	0.137	0.119	86.8	2.34	1.80
50	10.0	0.325	0.288	88.8	2.56	1.96
50	20.0	0.625	0.526	76.4	2.36	1.31
100	0.0	0.020	0.009	45.0	4.49	3.33
100	0.001	0.020	0.010	50.0	4.33	3.19
100	0.01	0.025	0.012	48.0	3.69	2.78
100	0.10	0.029	0.020	68.9	3.39	2.15
100	0.25	0.040	0.022	55.0	4.09	3.18
100	5.0	0.137	0.102	74.4	3.77	2.52
100	10.0	0.325	0.267	82.1	4.36	3.37
100	20.0	0.749	0.650	86.7	4.42	3.26
250	0.0	0.020	0.009	45.0	5.39	2.76
250	0.001	0.025	0.012	40.0	5.06	3.36
250	0.01	0.025	0.015	60.0	4.82	3.49
250	0.10	0.030	0.019	63.3	3.95	3.04
250	0.25	0.040	0.022	55.0	4.97	3.70
250	5.0	0.137	0.111	81.0	4.70	3.40
250	10.0	0.325	0.263	80.9	5.60	4.32
250	20.0	0.750	0.608	81.0	5.11	3.58
500	0.0	0.025	0.010	40.0	5.56	4.62
500	0.001	0.025	0.010	40.0	5.27	3.84
500	0.01	0.030	0.019	63.3	5.76	3.38
500	0.10	0.040	0.021	52.5	6.07	3.38
500	0.25	0.040	0.025	62.5	5.15	4.04
500	5.0	0.175	0.139	79.4	5.59	3.92
500	10.0	0.325	0.255	78.4	5.55	4.15
500	20.0	0.625	0.447	71.5	9.25	5.84

fluenced by the calcium level. It is also evident from table 5 that, as was found in Series I, the soluble boron within the plant was somewhat less at the highest calcium level (500.0 p.p.m.) than at all of the lower nutrient levels of calcium. As in the first series, the data also show that in most cases a relatively higher percentage of the total boron remains soluble in the corn plant at the high boron levels than at lower nutrient levels of boron. However, the data fail to explain the qualitative observations made earlier with

TABLE NO. 6

RATIOS OF DRY WEIGHT TISSUE CONTENTS OF TOTAL CALCIUM TO TOTAL BORON OF WHOLE TOPS OF CORN PLANTS OF SERIES II

Treatment		Total Ca / Total B	Treatment		Total Ca / Total B
Ca	B		Ca	B	
p. p. m.	p. p. m.		p. p. m.	p. p. m.	
5	0.0	31	100	0.0	228
5	0.001	24	100	0.001	218
5	0.01	15	100	0.01	148
5	0.10	17	100	0.10	119
5	0.25	15	100	0.25	103
5	5.0	3	100	5.0	27
5	10.0	2	100	10.0	13
5	20.0	0.9	100	20.0	6
10	0.0	40	250	0.0	271
10	0.001	33	250	0.001	203
10	0.01	29	250	0.01	194
10	0.10	26	250	0.10	132
10	0.25	26	250	0.25	125
10	5.0	4	250	5.0	34
10	10.0	3	250	10.0	17
10	20.0	1	250	20.0	7
50	0.0	115	500	0.0	223
50	0.001	94	500	0.001	212
50	0.01	88	500	0.01	193
50	0.10	74	500	0.10	152
50	0.25	53	500	0.25	129
50	5.0	17	500	5.0	32
50	10.0	8	500	10.0	17
50	20.0	6	500	20.0	15

regard to boron deficiency, that is, that this injury is intensified with increasing concentrations of calcium in the substrate, nor does it satisfactorily explain why boron toxicity decreased with increasing concentrations of calcium in the substrate. In tomato plants (4,37), the qualitative observations are in agreement with the quantitative data with regard to boron deficient and boron toxic plants. An explanation for this lack of agreement in the corn plant will be given later when the quantitative relationship between calcium and boron in the plant is considered, together with the analytical data with respect to total and soluble boron of the fractionated portions of the plant.

From the results presented with regard to total and soluble calcium of the plant, it is evident that the same trend follows as with the results obtained for the first series, that is, that the calcium content in the tissues is largely determined by the calcium concentration of the growth medium and is not greatly influenced by boron, with one principal exception, namely, in the case of plants grown at 500.0 p.p.m. calcium and 20.0 p.p.m. boron where total and soluble calcium content was much higher as compared with that of plants grown at the same calcium level but different boron concentrations. With the exception mentioned above, similar results were obtained

TABLE NO. 7

TOTAL AND SOLUBLE BORON AND CALCIUM IN MILLIGRAMS PER GRAM DRY TISSUE OF *TOP LEAVES* OF CORN PLANTS OF SERIES II

Treatment		Total	Soluble	Total	Soluble
Ca	B	B	B	Ca	Ca
p. p. m.	p. p. m.	mgm. p. g.	mgm. p. g.	mgm. p. g.	mgm. p. g.
5	0.0	0.040	0.014	0.22	0.003
10	0.0	0.025	0.012	0.76	0.04
50	0.0	0.022	0.012	1.33	0.38
100	0.0	0.025	0.005	2.47	0.58
250	0.0	0.012	0.001	3.04	2.17
500	0.0	0.019	0.004	4.46	2.87
5	0.25	0.044	0.030	0.52	0.03
10	0.25	0.044	0.028	0.78	0.37
50	0.25	0.045	0.028	2.45	1.62
100	0.25	0.045	0.026	4.04	2.82
250	0.25	0.045	0.028	3.81	2.06
500	0.25	0.045	0.031	3.05	1.85
5	20.0	0.624	0.437	0.25	0.09
10	20.0	0.624	0.391	0.56	0.27
50	20.0	0.624	0.295	1.85	0.95
100	20.0	0.874	0.727	2.33	1.38
250	20.0	0.874	0.696	2.95	1.71
500	20.0	0.873	0.743	4.02	2.45

by Warington working with *Vicia faba* (47), Reeve (36), Brennan (4) working with tomato, and Reeve and Shive (37) working with both corn and tomato plants.

Again the ratios of total calcium to total boron in the plants were calculated and are presented in table 6. As in Series I, somewhat similar differences in ratios were obtained. It is evident from table 6 that, in general, at a given boron level as the calcium concentration in the substrate increase, the ratio of Ca/B increases also, while at a given calcium level as the boron in the substrate increases, the ratio decreases. Plants showing boron deficient symptoms had rather high Ca/B ratios especially at the higher calcium concentrations in the substrate, whereas plants with relatively intense boron-toxic symptoms had a very low ratio at the low calcium concentrations as compared with the intermediate ratios of healthy plants. As with the first corn series, the ratios presented indicate that a certain balance must exist between the calcium and boron content of the corn tissue to fulfill the requirements of healthy individuals. The fact that boron deficient and boron toxic plants had a high and low Ca/B ratio, respectively, points to the importance of the relationship between these two elements within the plant. The data of table 6 show that at the highest boron level (20.0 p.p.m.) as the calcium concentration of the substrate was increased the Ca/B ratio increased, as was to be expected, and is considerably higher at the last four calcium levels (50.0, 100.0, 250.0, 500.0 p.p.m. calcium) than at the first two calcium levels, respectively, (5.0,

TABLE NO. 8

TOTAL AND SOLUBLE BORON AND CALCIUM IN MILLIGRAMS PER GRAM DRY TISSUE OF *LOW LEAVES* OF CORN PLANTS OF SERIES II

Treatment		Total	Soluble	Total	Soluble
Ca	B	B	B	Ca	Ca
p. p. m.	p. p. m.	mgm. p. g.	mgm. p. g.	mgm. p. g.	mgm. p. g.
5	0.0	0.030	.005	1.16	0.48
10	0.0	0.025	.015	1.43	0.62
50	0.0	0.025	.014	3.80	1.63
100	0.0	0.025	.008	4.83	3.05
250	0.0	0.022	.012	7.61	5.21
500	0.0	0.040	.023	9.13	6.88
5	0.25	0.056	.037	0.96	0.61
10	0.25	0.044	.025	1.35	0.55
50	0.25	0.045	.025	4.59	3.41
100	0.25	0.045	.027	5.48	4.03
250	0.25	0.045	.028	8.72	6.25
500	0.25	0.045	.028	7.77	5.81
5	20.0	1.750	1.585	0.91	0.52
10	20.0	0.874	.737	0.88	0.49
50	20.0	0.874	.455	4.01	2.46
100	20.0	1.130	.970	4.67	3.32
250	20.0	1.500	1.342	7.52	5.73
500	20.0	1.500	1.335	10.33	8.12

10.0 p.p.m. calcium). It was at these last four calcium concentrations that the plants showed a decrease in the intensity of injury due to boron toxicity, as can be observed in figure 8. The less toxic boron plants had a Ca/B ratio of 7 and 15 respectively as compared with ratios of 0.9 and 1 respectively of those plants showing much more boron toxicity. Thus it is apparent that there seems to exist a direct association between the appearance of boron deficiency and toxicity symptoms in the corn plant and the quantitative relationships of calcium and boron within the plant.

Results of Chemical Analyses of Tissue Fractions.—The analytical results of quantitative tests for total and soluble boron and total and soluble calcium of the tissues of the fractionated plants are presented in tables 7, 8, 9, and 10. The results obtained bring out several interesting points with regard to total and soluble boron content of the various tissues at the different boron levels. The tables show that, in general, at the lowest boron concentration of the substrate there was a greater accumulation of total and soluble boron in the older tissues of the plant (top stems, low stems, and low leaves) than in the top leaves and these differences were particularly marked at the highest nutrient levels of calcium. At the lowest nutrient level of boron the soluble boron contents of the top leaves at the three highest calcium levels (100.0, 250.0, and 500.0 p.p.m.) are relatively low as compared with the values at the three lower calcium levels. These data are in agreement with the qualita-

TABLE NO. 9

TOTAL AND SOLUBLE BORON AND CALCIUM IN MILLIGRAMS PER GRAM DRY TISSUE OF *TOP STEMS* OF CORN OF SERIES II

Treatment		Total	Soluble	Total	Soluble
Ca	B	B	B	Ca	Ca
p. p. m.	p. p. m.	mgm. p. g.	mgm. p. g.	mgm. p. g.	mgm. p. g.
5	0.0				
10	0.0	0.030	0.011	0.75	0.41
50	0.0	0.030	0.013	1.42	0.99
100	0.0	0.030	0.009	1.78	1.13
250	0.0	0.040	0.017	2.93	1.88
500	0.0	0.050	0.016	3.51	2.59
5	0.25	0.050	0.023	0.44	0.17
10	0.25	0.045	0.027	0.50	0.26
50	0.25	0.030	0.012	1.34	0.85
100	0.25	0.040	0.013	2.00	1.36
250	0.25	0.040	0.015	2.45	1.68
500	0.25	0.050	0.019	4.55	1.64
5	20.0	0.125	0.087	0.25	0.01
10	20.0	0.350	0.307	0.55	0.11
50	20.0	0.299	0.261	1.89	1.32
100	20.0	0.225	0.302	2.73	1.96
250	20.0	0.274	0.218	7.01	5.50
500	20.0	0.225	0.178	6.41	4.99

tive observations made at the time of harvest, when it was noted that the boron-deficiency symptoms of the top leaves of the corn plant were most severe at the three highest calcium levels. However, it should be mentioned that the boron deficient injury was more pronounced in the plants supplied with 250.0 p.p.m. calcium than at either of the other two highest calcium levels. Thus, it is evident, that symptoms of boron deficiency are associated with the presence of small amounts of boron in the top portions of boron deficient plants as compared with plants receiving adequate amounts of boron (9). It appears from the results presented that at low boron concentration, a large proportion of boron accumulates in the older tissues of the plants and is not freely translocated to the top leaves where synthetic activity is highest. The fact that a large part of the total and soluble boron in plants to which boron was not intentionally supplied accumulated in the lower or older tissues instead of in the top leaves was probably responsible for the lack of agreement between the qualitative observations of boron deficient plants made at the time of harvest and quantitative results of the same plants when a composite sample of the whole top was analyzed for total and soluble boron. Unquestionably the low total and soluble boron content of the top leaves was masked by the presence of tissues containing high total and soluble boron (top stems, low leaves, and low stems) in the composite sample and, therefore, the qualitative observations of the boron deficient plants were not confirmed by quantitative analysis of whole tops. This indicates that the values

TABLE No. 10

TOTAL AND SOLUBLE BORON AND CALCIUM IN MILLIGRAMS PER GRAM DRY TISSUE OF *LOW STEMS* OF CORN PLANTS OF SERIES II

Treatment		Total	Soluble	Total	Soluble
Ca	B	B	B	Ca	Ca
p. p. m.	p. p. m.	mgm. p. g.	mgm. p. g.	mgm. p. g.	mgm. p. g.
5	0.0	0.020	0.004	0.29	0.13
10	0.0	0.045	0.017	0.54	0.30
50	0.0	0.045	0.030	1.26	0.89
100	0.0	0.020	0.008	3.36	2.86
250	0.0	0.030	0.013	4.05	3.22
500	0.0	0.040	0.012	6.86	5.58
5	0.25	0.022	0.008	0.28	0.15
10	0.25	0.022	0.008	0.35	0.21
50	0.25	0.034	0.014	1.56	1.19
100	0.25	0.045	0.024	2.88	2.29
250	0.25	0.045	0.020	3.46	2.66
500	0.25	0.040	0.016	5.54	4.20
5	20.0	0.175	0.161	0.29	0.03
10	20.0	0.350	0.313	0.34	0.15
50	20.0	0.349	0.309	2.01	1.46
100	20.0	0.274	0.246	2.41	1.92
250	20.0	0.349	0.304	6.99	5.97
500	20.0	0.350	0.302	13.33	11.29

of total and soluble boron contents of boron deficient plants as a whole do not provide reliable criteria of the concentration present in any given tissue.

The plants of the cultures supplied with 0.25 p.p.m. boron showed a much more equal distribution of this element throughout the various tissues than plants supplied with no boron or with very high nutrient levels of this element. At the nutrient level of 0.25 p.p.m. boron there was relatively little variation in the total boron contents of any given tissue fraction of plants grown with different calcium levels. At the same nutrient level of boron (0.25 p.p.m.) the soluble boron was higher in the top leaves and lower leaves than in the top and lower stems of the plants at any given calcium level, except in one case. This might be expected, since the leaves are more active than the stems in metabolic processes in which boron must take part.

The analytical data for total and soluble boron of all the cultures supplied with 20.0 p.p.m. boron show that the distribution of boron in the plant followed a definite course (19). The greatest amount of total and soluble boron accumulated in the lower leaves, while a considerably lesser amount was found in the top leaves. Low stems had a higher boron content than top stems. The qualitative observations made in regard to boron toxicity symptoms of plants grown at the highest boron level are in agreement with the quantitative data

TABLE NO. 11

RATIOS OF DRY WEIGHT TISSUE CONTENTS OF TOTAL CALCIUM TO TOTAL BORON IN THE VARIOUS FRACTIONS OF THE CORN PLANTS OF SERIES II

Treatment		Top Leaves Total Ca / Total B	Low Leaves Total Ca / Total B	Top Stems Total Ca / Total B	Low Stems Total Ca / Total B
Ca	B				
p. p. m.	p. p. m.				
5	0.0	5	38	.....	15
10	0.0	31	57	25	12
50	0.0	59	153	47	28
100	0.0	99	194	60	169
250	0.0	245	340	73	135
500	0.0	224	229	70	172
5	0.25	12	17	9	13
10	0.25	18	31	11	16
50	0.25	55	102	45	46
100	0.25	90	123	50	65
250	0.25	85	194	62	77
500	0.25	68	173	91	139
5	20.0	0.4	0.5	2	2
10	20.0	0.9	0.7	2	1
50	20.0	3.0	5.0	6	6
100	20.0	3.0	4.0	12	9
250	20.0	3.0	5.0	25	20
500	20.0	5.0	7.0	29	38

presented. The higher concentration of boron in the lower leaves paralleled the presence of boron toxic effects as evidenced by the brown and dead margins and tips of the lower leaves of the plants. Similar results were obtained by Eaton (8) and Purvis (34) due to a great accumulation of boron in the lower leaves of the plants.

The data further show that the total and soluble boron content of the various tissues depended largely upon the boron concentration of the substrate. However, in some cases the relative calcium concentration of the substrate modified the accumulation of this element within the plant at a given boron level, the greatest modification occurring at the highest boron level. As a general rule, the leaves were more sensitive to boron changes in the substrate than were either the top or low stems. Parks, Lyons, and Hood (31) reported that as boron supply in the substrate increased, the concentration of boron in the leaves increased significantly.

Calcium Content of Plant Tissues.—The analytical evidence gathered with reference to the calcium content of the various tissues of the corn plant shows that its distribution is regular and definite. The greatest amount of total and soluble calcium was found in the leaves of the plant, the lower leaves having the largest amount of this element (45). In general, the lower stems had a much higher calcium content than the top stems. The fact that much more total

and soluble calcium was found in the lower leaves and lower stems suggests that most of it was tied up in this lower portion of the plant and, therefore, was not freely translocated to the younger tissue where meristematic activity is highest. As a result of this condition the severe calcium deficiency symptoms of the cultures grown at 5.0 p.p.m. calcium and less marked deficiency symptoms at 10.0 p.p.m. calcium can be explained. It is interesting to note from table 7 that the top leaves of those plants grown at these two lowest calcium levels without boron contained considerably less soluble calcium than the top leaves of those plants grown at the same calcium levels but having boron in the substrate. The total calcium of the top leaves of the calcium deficient plants (5.0, 10.0 p.p.m. calcium) receiving no boron and 20.0 p.p.m. boron respectively at each of the two lowest calcium levels are comparable. There were only little differences between the total calcium contents of the top leaves of the plants grown at the lowest boron level (0.0 p.p.m.) and the

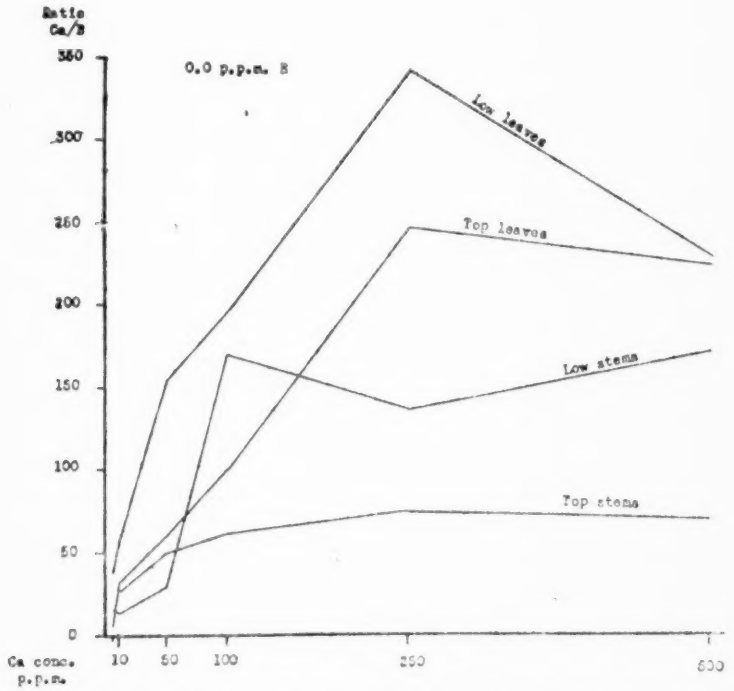

FIG. 9.—Ratios of total calcium to total boron in each fraction of corn plants grown at 0.0 p.p.m. boron plotted against calcium concentration in the substrate.

highest boron level (20.0 p.p.m.) at either of the two lowest nutrient levels of calcium respectively (5.0, 10.0 p.p.m.). This, however, was not true in the case of the soluble calcium content of these same tissues; the soluble calcium content being considerably greater in the calcium deficient plants grown at the highest boron level. These differences may be associated with the soluble boron content of the tissues of these calcium deficient plants. Where the soluble calcium content of the tissue was greatest, it was found that the soluble boron content was also the greatest. In other words, the soluble calcium in the top leaves of these calcium deficient plants is not so much a function of the total calcium as it is of the soluble boron. This relation described above did not hold true in the case of the other plant fractions. The same relation between the tissue content of soluble calcium and soluble boron has been obtained by Marsh and Shive (17) for whole tops of calcium deficient corn plants, by Lowenhaupt with the sunflower (16), and by Lorenz with garden beet (15). In general, at a given boron level as the calcium concentration of the substrate increased, there resulted an increased accumulation of total and soluble calcium in each of the various fractionated portions of the plant (47). In general, at a given calcium level, variations in the boron concentrations of the substrate did not influence greatly the calcium content of the tissue of the various plant fractions, except at the two highest calcium levels in the case of the top stems and lower stems where the values of total and soluble calcium were very high at the highest boron level.

From the analytical data pertaining to the calcium and boron content of the various tissues of the plants grown at various nutrient treatments, the Ca/B ratios were calculated in a manner similar to that previously used to determine Ca/B ratios for the whole tops. These ratios are presented in table 11. It is evident that plants showing boron deficient symptoms had the highest Ca/B ratios in the various fractionated portions while those giving evidence of boron toxicity and calcium deficiency had very low Ca/B ratios. On the other hand, the plants which did not show any injury and which were apparently healthy had intermediate ratios. The plants which had the greatest green and dry weights, those supplied with 250.0 p.p.m. calcium and 0.25 p.p.m. boron, and which were in all respects healthy, had a ratio of Ca/B of 125 as calculated from the composite sample of the whole tops (table 6). The Ca/B ratios of the individual tissue fractions of the plants grown with the same nutrient treatments were as follows: top leaves 85; low leaves 194; top stem 62; and low stem 77 (table 11). As was to be expected,

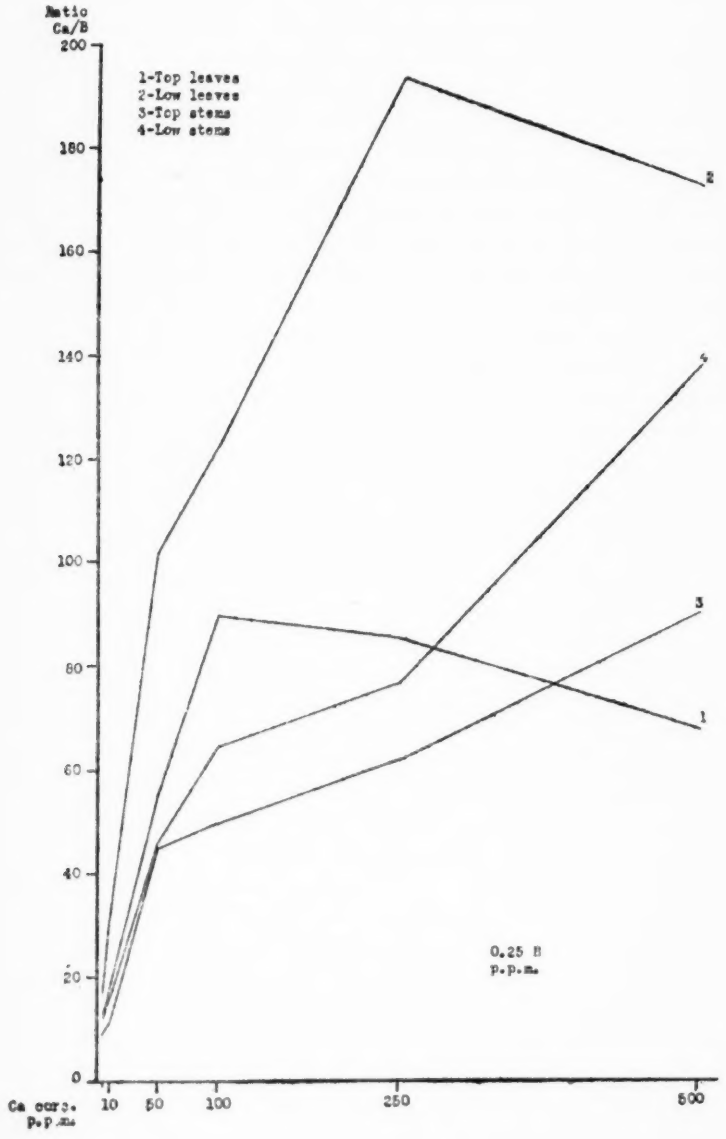

FIG. 10.—Ratios of total calcium to total boron in each fraction of corn plants grown at 0.25 p.p.m. boron plotted against calcium concentration in the substrate.

because of the greater accumulation of calcium and boron in the older tissues of the plant, the Ca/B ratios of the lower leaves were higher than those of the top leaves in all but one case. The same trend followed with regard to the Ca/B ratios of the lower stem and the top stem fractions except that at the highest boron concentration there was not much difference between the Ca/B ratios of the top and the low stem tissues respectively at any nutrient level of calcium except the highest (500.0 p.p.m.).

As can be seen from table 11 and figures 9, 10, 11, 12 and 13, as the calcium increased at a given boron level, the Ca/B ratio increased in the majority of cases. On the other hand, as the boron increased at a given calcium level, the ratio decreased.

From the analytical results and Ca/B ratios presented for both series of corn, it is evident that a certain relationship exists between calcium and boron which affects the metabolism of the corn plant, but which, however, is not so striking as with the tomato (4, 37). The quantitative results obtained in the second corn series for the total and soluble boron and calcium contents of the composite samples did not confirm the qualitative observations made, namely, that the boron deficiency symptoms were increasingly severe with increasing calcium concentration of the substrate. However, when a

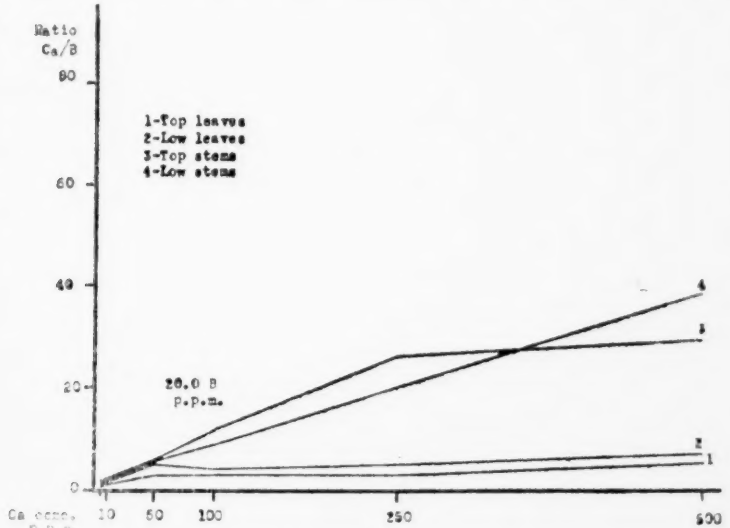

FIG. 11.—Ratios of total calcium to total boron in each fraction of corn plants grown at 20.0 p.p.m. boron plotted against calcium concentration in the substrate.

representative plant from the culture supplied with no boron at the highest calcium levels was fractionated, the analytical results for total and soluble boron for the top leaves were in agreement with the qualitative observations made on the tops of these boron deficient plants. Since a considerable amount of total and soluble boron of these boron deficient plants was found in the older tissues as compared with the top leaves, probably this was the reason why the analytical data of the composite samples did not corroborate the qualitative observations. The higher total and soluble boron of these fractions masked the small amount of total and soluble boron of the top leaves when all the tissues were mixed to take the composite sample, and, therefore, little or no difference was evident between boron contents of whole top samples of different nutrient treatments. The results further suggest that there is probably little

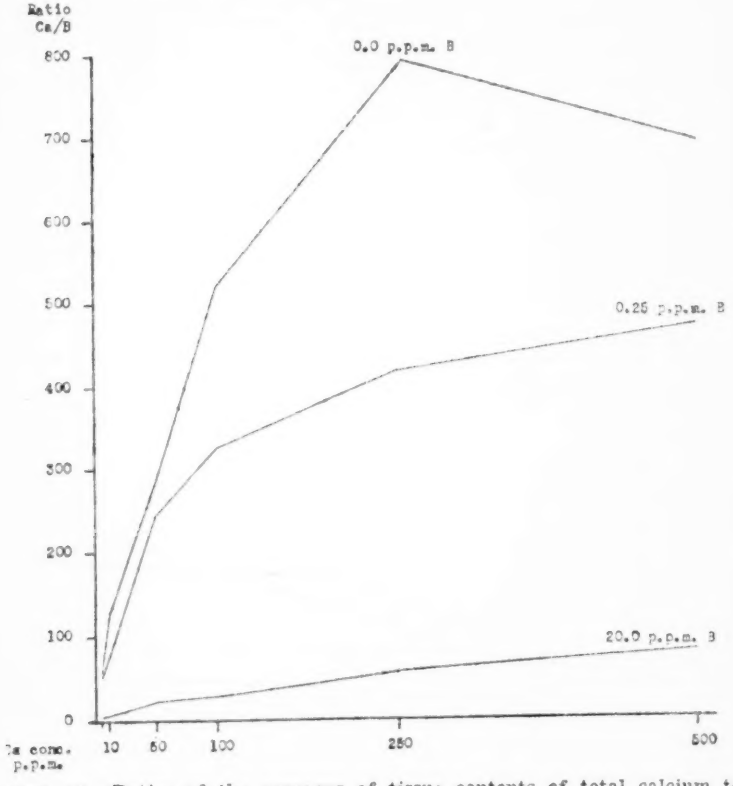

FIG. 12.—Ratios of the averages of tissue contents of total calcium to total boron of the four different plant fractions of corn plants grown at nutrient levels of 0.0, 0.25, and 20.0 p.p.m.

if any translocation of soluble boron from the older tissues of the boron deficient plants to the top leaves where meristematic activity is at its maximum. It should be mentioned at this point that it took a considerable time for the boron deficiency symptoms to appear in these cultures in both series. However, it took less time than in the similar cultures grown in the fall season (46). The fact that the boron requirements for corn are rather low (18) and that a relatively large proportion of this element is in the soluble state in the plant tissue may explain the late appearance of the injury due to boron deficiency in the top leaves and roots. This also suggests why corn plants made fairly good growth up to the time when the deficiency symptoms appeared. It is probable that the late appearance of boron deficiency symptoms in plants, to which boron was not intentionally supplied, was due in part to the presence of boron as impurities in the salts used. In fact, it was found necessary to repurify one of the salts, namely, $CaCl_2$ for the second series, by recrystalization.

As was pointed out previously, the Ca/B ratios of the boron deficient plants were high. Plants that received no boron in the substrate and which were supplied with calcium concentrations of 5.0, 10.0, and 50.0 p.p.m. had a much lower Ca/B ratio than those

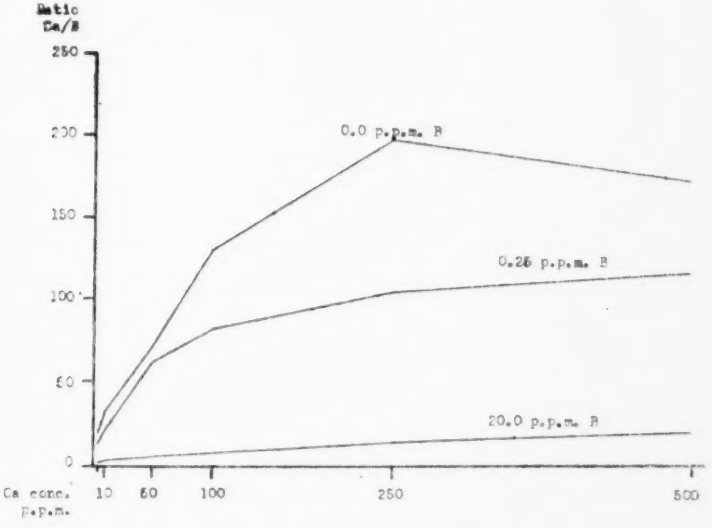

FIG. 13.—Ratios of total calcium to total boron of whole tops of corn plants grown at boron levels of 0.0, 0.25, and 20.0 p.p.m. plotted against calcium concentration in the substrate.

at the highest calcium nutrient levels and showed either slight boron deficiency symptoms or no symptoms at all. It appears that the combination of high calcium and deficient boron in the substrate upsets the normal metabolism of the plant more seriously than a combination of low calcium and deficient boron.

Neither the quantitative data for total and soluble boron of the composite nor the fractionated portions of the plant confirmed completely the qualitative observations made with regard to a decrease of boron toxicity in the plants supplied with 20.0 p.p.m. boron as the calcium concentration of the substrate increased. On the other hand, the calculated Ca/B ratios for the composite and fractionated portions (table 6 and table 11) of the plants furnish some interesting information. It is in the quantitative relationship between the calcium and boron within the plant that the decrease in the severity of boron toxic symptoms can be associated. The plants that showed relatively mild boron toxicity symptoms, which were the plants grown with the highest calcium concentrations of the substrate, had higher Ca/B ratios than those showing more severe boron toxicity symptoms. This indicates that a combination of high calcium and high boron in the substrate tends to favor a more normal metabolism of the plant than a combination of low calcium and high boron, which brings about a disturbance in the metabolic activities of the plant.

The plants grown in the substrate containing 0.01 to 0.25 p.p.m. boron were devoid of any boron deficiency or boron toxicity symptoms, thus indicating that their metabolism was normal. These plants had Ca/B ratios which were intermediate between those of boron-deficient and boron-toxic plants.

The quantitative results presented in this report for total and soluble calcium in the corn plant definitely show that the quantitative relationship between these two elements within the plant plays a very important role in the metabolic activities of the plant. Moreover, the data show that a certain balance between calcium and boron must exist within the plant to fulfill the requirements for normal growth and development of this monocotyledon.

SUMMARY

Corn plants were grown in sand culture using the continuous flow method with six different kinds of nutrient solutions which contained different calcium and boron concentrations. A qualitative and quantitative study was made of the response of the plants to the different calcium and boron levels. The results may be summarized as follows:

1. Plants showing symptoms of boron deficiency were found by chemical analyses to have a low content of total and soluble boron and those showing symptoms of boron toxicity a very high content of total and soluble boron.

2. Boron deficient plants were characterized by the appearance of elongated, white transparent stripes in the newly formed leaves. The growth of boron toxic plants was stunted, their top leaves yellow or yellow-green and margins and tips of lower leaves brown and dead.

3. Composite samples of the whole tops of plants showed by analysis that both total boron and soluble boron were largely independent of the calcium concentration in the substrate, except that soluble boron content of the plants grown with the highest boron level (20.0 p.p.m.) was less when supplied with nutrient levels of 500.0 p.p.m. calcium than with the lower calcium levels.

4. The accumulation of calcium in the composite tissue samples of the tops was found to be largely determined by the calcium concentration in the substrate, except that at the highest nutrient level of boron (20.0 p.p.m.) the calcium content of the tissue of plants grown with high nutrient levels of calcium (100.0, 250.0, 500.0 p.p.m.) was higher than those of plants grown at lower nutrient levels of boron.

5. Boron deficiency symptoms increased in severity with increasing calcium concentration of the substrate throughout the range of the four highest nutrient levels of calcium, except that somewhat more intense boron deficiency symptoms were detected at 250.0 p.p.m. calcium than at 500.0 p.p.m. calcium concentration. Top leaves of boron deficient plants grown at 0.0 p.p.m. boron and which showed boron deficiency symptoms had, in general, lower total and soluble boron content than top leaves of plants grown at the same boron

level but at the two lowest calcium levels which did not exhibit boron deficiency symptoms. The older tissues of these plants showing external boron deficiency symptoms had a considerably higher total and soluble boron content than the younger tissues (top leaves).

6. The results of this study indicate that there is a limited rate of translocation of boron from the lower portions to the top leaves of boron deficient plants.

7. Boron toxicity decreased in severity with increasing calcium concentration of the substrate.

8. Increasing the concentration of boron in the culture solution resulted in a marked increase of total and soluble boron in all tissues of the plant.

9. There was a greater accumulation of calcium and boron in the older tissues of the plant than in the tissue where meristematic activity was highest.

10. The calcium content of the tissues was determined to a large degree by the calcium concentration of the substrate and was largely independent of the nutrient level of boron.

11. Differences in the soluble calcium contents of the top leaves of calcium deficient plants were related to the soluble boron content of the tissues, which in turn were determined by the boron concentration of the substrate.

12. The quantitative relationship between calcium and boron within the plant plays an important role in the metabolic activities of the plant. Boron deficient plants had a high Ca/B ratio, while boron toxic plants had a very low ratio. Plants which apparently were healthy in all respects had intermediate ratios.

13. At a given boron level, increments in the calcium concentration in the substrate caused an increase in the Ca/B ratio of the tissues.

14. At a given calcium level, increments in the boron concentration in the growth media caused a decrease in the Ca/B ratio of the tissues.

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